

BRIEFING PACKAGE

Division of Anti-Infective Products
Office of Antimicrobial Products
Center for Drug Evaluation and Research, FDA

BLA 125349

RAXIBACUMAB

APPLICANT: HUMAN GENOME SCIENCES, INC.

ANTI-INFECTIVE DRUGS ADVISORY COMMITTEE MEETING

NOVEMBER 2, 2012

**PROPOSED INDICATION:
TREATMENT OF INHALATIONAL ANTHRAX**

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TABLE OF CONTENTS

1.	INTRODUCTION.....	4
1.1	BACILLUS ANTHRACIS AND ANTHRAX DISEASE	5
1.2	CURRENTLY AVAILABLE ANTHRAX THERAPIES.....	6
1.3	UNMET MEDICAL NEED	6
2.	REGULATORY BACKGROUND.....	6
2.1	BIOLOGIC LICENSING APPLICATION (BLA).....	6
2.2	ANIMAL RULE (NEW DRUGS AND BIOLOGICS).....	10
2.2.1	<i>Criteria for Submission.....</i>	<i>10</i>
2.2.2	<i>Criteria for Approval.....</i>	<i>11</i>
3.	MICROBIOLOGY OF ANTHRAX AND RAXIBACUMAB ACTIVITY.....	12
3.1	BACILLUS ANTHRACIS	12
3.2	RAXIBACUMAB.....	12
3.1.1	<i>Mechanism of Action</i>	<i>13</i>
3.1.2	<i>Activity in vivo</i>	<i>13</i>
4.	ANTHRAX DISEASE – NATURAL HISTORY IN ANIMAL MODELS AND COMPARISON TO HUMAN DISEASE.....	14
5.	NEW ANIMAL EFFICACY STUDIES (2012)	18
5.1	CNS TOXICITY STUDY IN RABBITS	18
5.1.1	<i>Study Design.....</i>	<i>18</i>
5.1.2	<i>Results.....</i>	<i>20</i>
5.1.3	<i>Necropsy and Histopathology.....</i>	<i>25</i>
5.2	ADDED BENEFIT STUDY FOR RAXIBACUMAB IN RABBITS	31
5.2.1	<i>Study Design.....</i>	<i>31</i>
5.2.2	<i>Results.....</i>	<i>32</i>
5.2.3	<i>Necropsy and Histopathology.....</i>	<i>37</i>
6.	CLINICAL PHARMACOLOGY	40
6.1	INTERSPECIES COMPARISON OF RAXIBACUMAB PHARMACOKINETICS	40
6.2	DRUG INTERACTIONS	43
6.3	EXPOSURE-RESPONSE FOR EFFICACY	45
7	SAFETY.....	51
7.1	NONCLINICAL PHARMACOLOGY/TOXICOLOGY SAFETY	51
7.1.1	<i>Tissue Cross-Reactivity of Raxibacumab.....</i>	<i>51</i>
7.1.2	<i>General Toxicity of Raxibacumab in Healthy Cynomolgus Monkeys.....</i>	<i>52</i>
7.1.3	<i>Reproductive Toxicology of Raxibacumab in Healthy New Zealand White Rabbits</i>	<i>52</i>
7.2	CLINICAL SAFETY	52
7.3	IMMUNOGENICITY	57
8	POINTS FOR DISCUSSION	58

1. Introduction

On November 2, 2012, the Anti-Infective Drugs Advisory Committee will meet to discuss the re-submission of the Biological Licensing Application (BLA) 125,349 for raxibacumab. The proposed indication for raxibacumab is the treatment of inhalational anthrax. Raxibacumab would be administered in a 40 mg/kg single 2-hour intravenous infusion. Human Genome Sciences, Inc. (HGS) submitted the original BLA 125,349 for raxibacumab in 2009 and raxibacumab was previously discussed in an Anti-Infective Drugs Advisory Committee (AIDAC) meeting¹ in October 2009. HGS re-submitted the BLA for raxibacumab in June 2012. This briefing document will summarize the information provided by HGS in the original BLA, as well as describing the new animal studies provided in the re-submission.

Inhalational anthrax is caused by the exotoxin-producing Gram-positive bacterium, *Bacillus anthracis*. While antibacterial treatment is directed at *B. anthracis* eradication, it has no activity against the toxins produced by *B. anthracis*: lethal toxin (LT) and edema toxin (ET). These toxins are formed when *B. anthracis* elaborates the components needed to form these toxins: protective antigen (PA), lethal factor (LF) and edema factor (EF).

HGS undertook the development of raxibacumab, a recombinant, fully human, IgG1 λ monoclonal antibody directed at the PA of *B. anthracis*, as an addition to the available treatment armamentarium for patients with inhalational anthrax. The development program included studies showing that raxibacumab binds PA with high affinity and inhibits PA binding to anthrax toxin receptor (ATR) on host cells, thereby protecting the cells from anthrax toxin-mediated injury. Proof-of-concept studies with raxibacumab demonstrated a greater proportion of surviving animals in the rat lethal toxin infusion model and in several pre-exposure and post-exposure prophylaxis animal model studies. The raxibacumab development program also included identification and characterization of the natural history of anthrax disease in animal models (New Zealand White rabbits and cynomolgus macaques). These animal models were used to evaluate the efficacy of raxibacumab in the treatment of inhalational anthrax.

Efficacy studies were conducted in animal models of infection because naturally-occurring inhalational anthrax infection in humans is rare and intentional exposure of healthy human volunteers to aerosolized *B. anthracis* spores for research purposes is unethical. Information on the Animal Rule, which allows the FDA to rely on animal efficacy studies, is discussed in Section 2. The animal model studies submitted to support efficacy in the original BLA application are also described in section 2, while new animal studies are described in section 5.

The safety of raxibacumab in normal volunteers and preclinical results of raxibacumab testing are summarized in section 7. These investigations demonstrated that raxibacumab was associated with the development of infusion reactions (primarily rash); therefore, subsequent studies in human subjects and monkey efficacy studies included diphenhydramine pre-treatment. Immunogenicity was evaluated for this intravenous product, and the results suggest a low risk for immunogenicity.

¹ <http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/ucm125599.htm>

1.1 *Bacillus anthracis* and Anthrax Disease

1.1.1 *Bacillus anthracis* infection

B. anthracis is a Gram-positive bacillus that causes three forms of anthrax depending on the route of exposure: cutaneous, gastrointestinal (GI), and inhalational. Inhalational anthrax disease results from entry of the *B. anthracis* spores via the respiratory tract and deposition in the lung. In the alveolar spaces, macrophages phagocytose the spores and then migrate to regional lymph nodes. The organisms proliferate in the lymph nodes, rather than as a primary focus in the lungs². The appearance of organisms in the lymphatics draining the lungs and the establishment of infection in the intrathoracic lymph nodes always precedes the development of bacteremia after aerosol exposure. As the phagocytic capacity of the lymph node is overwhelmed, vegetative organisms pass through efferent lymphatics, infect successive nodes, and ultimately enter the blood stream through the thoracic duct.

In the macrophages, the spores germinate within phagosomes and produce anthrax toxin, which is comprised of three protein components: lethal factor (LF), edema factor (EF), and protective antigen (PA). Individually, these components are harmless; however, they combine to form noxious toxins.

1.1.2 Human Clinical Disease

B. anthracis is a bacterium found in nature and is historically associated with disease in the agricultural setting, and later, in the industrial settings (ragpickers' or woolsorters' disease³). Disease can be acquired by the cutaneous, gastrointestinal or inhalational routes. Cutaneous anthrax is generally characterized by a local eschar and responds well to antimicrobial treatment. Gastrointestinal and inhalational anthrax are generally associated with bacteremia, toxemia, and even with antimicrobial treatment, are often fatal. Natural disease was very rare in the 20th century, with the exception of two situations following aerosolized exposures.

Bacillus anthracis is considered a CDC Category A agent for bioterrorism that is easily disseminated, results in high mortality rates with potential significant public health and social consequences. *B. anthracis* spores are environmentally hardy, and thus, readily bioweaponized. The World Health Organization estimates that intentional release of 50 kg of *B. anthracis* over an urban population of 5 million would sicken 250,000 and kill 100,000. A US Congressional Office of Technology assessment analysis estimates that between 130,000 and 3 million deaths would follow the release of 100 kg of *B. anthracis*.

In 1979, accidental release of *B. anthracis* spores from a factory in Sverdlovsk, Russia, led to 66 deaths due to inhalational anthrax⁴, and in 2001, *B. anthracis* was disseminated through the US mail resulting in 22 cases of anthrax, including 11 cases of cutaneous and 11 cases of inhalational anthrax. All patients with cutaneous anthrax responded to treatment. Mortality in the US inhalational anthrax cases was 45% (5/11).⁵

² Passalacqua, KD and Bergman, NH, *Bacillus anthracis*: interactions with the host and establishment of inhalational anthrax. *Future Microbiology* (2006) 1(4): 397-415.

³ Brachman PS. Inhalational anthrax. *Ann NY Acad Sci* 1980; 353:83-93.

⁴ Abramova FA et al. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak in 1979. *Proc Natl Acad Sci USA* 1993; 90:2291-4.

⁵ Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg Infect Dis* 2001; 7:933-44. Barakat LA, Quentzel HL, Jernigan JA. Fatal inhalational anthrax in a 94-year-old Connecticut woman. *JAMA* 2002; 287:863-8.

Early diagnosis of inhalational anthrax is difficult and requires a high index of suspicion. Prior to the 2001 attacks, clinical information was confined to a series of 18 cases reported in the 20th century and the limited data from Sverdlovsk.

Inhalational anthrax has been described as a two-stage illness. The disease has a typical incubation period of 1-6 days and begins with relatively mild, flu-like symptoms such as malaise, fatigue and slightly elevated temperature. Some patients complain of dyspnea, cough, vomiting, chills, weakness, abdominal pain, and chest pain. Signs of illness and laboratory studies are nonspecific. This stage of illness lasts from hours to a few days. In some patients, a brief period of apparent recovery follows. Other patients progress directly to the second, fulminant stage of disease characterized by respiratory failure and shock. This second stage develops abruptly, with sudden fever, dyspnea, diaphoresis, and shock. Massive lymphadenopathy and expansion of the mediastinum lead to stridor in some cases. A chest radiograph most often shows a widened mediastinum consistent with lymphadenopathy. Up to half of patients develop hemorrhagic meningitis with concomitant meningismus, delirium, and obtundation. In this second stage, cyanosis and hypotension progress rapidly; death sometimes occurs within hours.

1.2 Currently Available Anthrax Therapies

Parenteral formulations of penicillin G and tetracycline class drugs (e.g., doxycycline, tetracycline, demeclocycline, and minocycline) are approved for treatment of anthrax due to *B. anthracis*. In addition, several antibacterial drugs include specific labeling for post exposure prophylaxis of inhalational anthrax (e.g. ciprofloxacin, levofloxacin, doxycycline, and penicillin G procaine).

Following the anthrax attacks of 2001, the CDC⁶ recommended the use of two or three antimicrobials in combination in persons with inhalational anthrax based on susceptibility testing with epidemic strains. Limited early information suggested that patients treated intravenously with two or more antimicrobials active against *B. anthracis* had a greater chance of survival.

1.3 Unmet Medical Need

Anthrax disease is associated with three principle virulence factors of *B. anthracis*: its antiphagocytic capsule and two protein exotoxins (lethal and edema toxins). Antimicrobial treatment results in eradication of the susceptible bacteria; however, antimicrobials have no activity against the toxins. There was a high mortality rate (45%) in inhalational anthrax cases during the 2001 US attack. Additional treatments that could improve the survival rate in patients with anthrax disease would address an unmet need.

2. Regulatory Background

2.1 Biologic Licensing Application (BLA)

The original BLA for raxibacumab was submitted to the FDA on May 14, 2009. The application contained a number of proof-of-concept studies in various animal models, safety pharmacology and clinical pharmacology studies in animals and humans, safety studies in healthy human volunteers, and four GLP animal efficacy studies of raxibacumab for the treatment of inhalational anthrax in New

⁶ Update: Investigation of Bioterrorism-Related Anthrax and Interim Guidelines for Exposure Management and Antimicrobial Therapy, October 2001 *MMWR*. 2001;50(42):909-19

Zealand white (NZW) rabbits and cynomolgus macaques as efficacy studies in humans were not considered ethical or feasible.

Efficacy Findings

NZW rabbits and cynomolgus macaques were selected for the efficacy studies of raxibacumab for treatment of inhalational anthrax. In the original BLA, four treatment studies with raxibacumab were submitted. The two efficacy studies tested raxibacumab versus placebo and two others evaluated the efficacy of raxibacumab plus antimicrobial versus antimicrobial alone (two studies each in rabbits and in monkeys). The animals were challenged with aerosolized *B. anthracis* spores at 200x LD₅₀. In rabbit studies sustained elevation of body temperature above baseline for 2 hours or a positive result on PA toxemia screen assay served as trigger for intervention (average 28 hours postexposure). In monkey studies intervention commenced at the time of positive PA result (average 42 hours postexposure). The review revealed that raxibacumab at 40 mg/kg IV single dose was superior to placebo in the rabbit and monkey raxibacumab monotherapy studies. In the monkey study, raxibacumab 20 mg/kg was also superior to placebo, but in the rabbit superior efficacy was limited to the 40mg/kg arm.

Table 2.1-1: Efficacy Results in Raxibacumab Monotherapy studies (FDA analysis)

Treatment group	Cynomolgus macaques at 28 days			NZW rabbits at 14 days		
	Number (%) of survivors	P value vs. placebo ¹	95% CI of raxibacumab-placebo ²	Number (%) of survivors	P value vs. placebo ¹	95% CI of raxibacumab-placebo ²
Placebo	0/10 (0%)			0/13 (0%)		
20 mg/kg raxibacumab	5/12 (41.7%)	0.0396	(7.2, 68.7)	4/16 (25.0%)	0.1067	(-2.2, 50.9)
40 mg/kg raxibacumab	9/13 (69.2%)	0.0016	(31.1, 88.9)	6/17 (35.3%)	0.0237	(7.3, 59.6)

¹ P value based on 2-sided Fisher's exact test for the comparison vs the placebo control group.

² 95% CIs are exact confidence intervals

When given in combination with antimicrobials, the efficacy of the combination was high, but the efficacy of antimicrobials alone was also high, raising the question whether the animal models adequately represented advanced anthrax disease in humans, where high mortality was observed despite antimicrobial treatment.

Table 2.1-2: Efficacy Results in Raxibacumab combination studies with antimicrobials (FDA analysis)

Treatment group	Cynomolgus macaques at 28 days		NZW rabbits at 28 days	
	Number (%) of survivors	95% CI of Cipro/raxibacumab-ciprofloxacin ²	Number (%) of survivors	95% CI of Levo/raxibacumab-levofloxacin ²
Placebo	0/10 (0%)	-	0/10 (0%)	-
Antimicrobial alone ¹	13/13 (100%)	-	19/20 (95.0%)	-
Antimicrobial ¹ + 40 mg/kg raxibacumab	11/13 (84.6%)	(-45.5, 11.4)	16/17 (94.1%)	(-23.9, 19.6)

¹ NHP study antimicrobial was 75 mg ciprofloxacin twice daily x 3 days

NZW rabbit study antimicrobial was 50 mg/kg levofloxacin daily x 3 days

² 95% CIs are exact confidence intervals

These animal studies were discussed in an AIDAC meeting in October of 2009. Based on the information from the natural history of inhalational anthrax disease in man, in rabbits, and monkeys; the mechanism of action of raxibacumab; and the efficacy of raxibacumab 40 mg/kg IV in the animal studies, FDA and the majority (16/24) of AIDAC committee members concluded that NZW rabbit and cynomolgus macaque models of inhalational anthrax disease are reflective of human disease and the efficacy findings from the therapeutic studies in these models could be extrapolated to humans.

However, several limitations of the efficacy findings in pivotal and combination animal studies and in animal models themselves were discussed.

First, the timing of intervention is likely to differ between animals and humans. In the experimental settings, animals were closely monitored for either an elevation in temperature (rabbits) or a positive plasma PA (rabbits and monkeys), and once detected, a therapeutic intervention was delivered. Humans may have delayed presentation to the healthcare setting or be given alternative diagnoses prior to the definitive diagnosis of anthrax being made. As the effects of delayed administration of raxibacumab after the onset of clinical symptoms has not been studied in the animal models, it is unclear how long raxibacumab administration can be delayed and still provide survival benefit.

Second, the combination efficacy studies demonstrated very high efficacies (nearly 100%) of levofloxacin in NZW rabbits and ciprofloxacin in monkeys, respectively. In contrast, during the 2001 anthrax attacks 5 of the 11 people who developed inhalational anthrax died despite best available antimicrobial and supportive treatment. In addition, a question regarding efficacy interaction between raxibacumab and antimicrobial when given together for the treatment of inhalational anthrax remained unanswered. Twelve of twenty four AIDAC committee members pointed out a number of deficiencies in the combination study design that precluded definite conclusions regarding efficacy interactions, e.g. timing of intervention, antibiotic regimen, and the absence of raxibacumab alone arm. A majority of the committee members (17/24) recommended additional evaluation of the contribution offered by raxibacumab to the efficacy of antimicrobial alone.

Third, the conditions of an animal experiment do not include any supportive care that human patients with inhalational anthrax will receive in a healthcare or clinical trial setting, including but not limited to mechanical ventilation, fluid resuscitation, pleural fluid drainage, dialysis, etc.

Fourth, due to concerns about the reliability of raxibacumab and antimicrobial PK data generated for the original BLA, FDA was unable to conclude that the proposed human dosing regimen would result in the exposures similar to those seen at the efficacious dose in animal studies. Because of these concerns, it was also unclear that the antimicrobial exposures in the combination animal studies did not exceed human exposures achieved with the approved human dose regimen. This concern has been addressed in the resubmission and the FDA now agrees that the pharmacokinetic data should be considered reliable (see Section 6.4).

Safety Findings

During the 2009 BLA review, an exaggerated inflammatory response in the CNS of the raxibacumab treated non-survivors compared to the placebo non-survivors was found on histopathological examination in the raxibacumab monotherapy animal studies. The duration of bacteremia prior to treatment / prior to death as well as time to death were similar between the placebo and raxibacumab non-survivors for both the monkey and the rabbit studies. However, within the raxibacumab groups, it appears the animals with CNS pathology lived longer than those without. Non-survivors in the raxibacumab 20 mg/kg dose group had a higher incidence and severity of CNS pathology as compared to the non-survivors the 40 mg/kg group, suggesting an absence of dose-response relationship for brain histopathology.

Table 2.1-3: Comparison of Histological Findings in Non-survivors by Treatment Group in NZW rabbits and cynomolgus macaques (monotherapy studies)

Organ/animal	Placebo (%)	Raxibacumab 20 mg/kg (%)	Raxibacumab 40 mg/kg (%)	P-value (20 vs. p)*	P-value (40 vs. p)*
Cynomolgus Macaque (As treated)					
Mortality	12/12 (100)	7/14 (50)	5/14 (35.7)	0.006	0.001
CNS	1/12 (8)	6/7 (86)	3/5 (60)	0.002	0.053
Liver	3/12 (25)	1/7 (14)	0/5 (0)	1.0	0.515
Spleen	11/12 (92)	5/7 (71)	4/5 (80)	0.523	0.515
Mediastinitis	10/11 (91)	4/7 (57)	4/5 (80)	0.245	1.0
Pneumonia	5/12 (42)	0/7 (0)	1/5 (20)	0.106	0.6
Nephritis	2/12 (17)	0/7 (0)	0/5 (0)	0.509	1.0
NZW Rabbit (As treated)					
Mortality	16/16 (100)	12/17 (70.5)	11/19 (57.9)	0.044	0.004
CNS	2/16 (12)	9/12 (75)	6/11 (55)	0.001	0.033
Liver	6/16 (38)	3/12 (25)	3/11 (27)	0.687	0.692
Spleen	11/16 (69)	2/12 (17)	2/11 (18)	0.009	0.018
Mediastinitis	15/16 (94)	7/12 (58)	8/11 (73)	0.057	0.273
Pneumonia	10/16 (63)	0/12 (0)	0/11 (0)	0.001	0.001
Nephritis	4/16 (25)	1/12 (8)	2/11 (18)	0.355	1.0
Cardiac	0/16 (0)	0/12 (0)	2/11 (18)	ns	0.157

two-sided Fisher's exact test
FDA analysis

These findings were presented at the AIDAC and their implications for humans were discussed. Additional studies to elucidate raxibacumab effect on CNS in survivors and non-survivors were recommended.

Animal Studies

One limitation of the pivotal efficacy studies with respect to the evaluation of the CNS findings was a lack of pathological examination of the survivors to allow comparison to the animals that died. In the monotherapy efficacy studies, surviving rabbits were euthanized and did not undergo necropsy, while surviving monkeys were observed for the duration of the study and confined to the Biosafety Level 3 (BL3) husbandry for 30 days post exposure, followed by a downgrade to a BL2 husbandry for an additional 60 days and then a release into the colony if in good health.

Although survivors were sacrificed in the combination levofloxacin/raxibacumab rabbit study, the high efficacy of the fluoroquinolone in this study limited the number of animals that died from anthrax in the active treatment arms. Only one animal in an active treatment group (levofloxacin alone) died of anthrax. Although it was reassuring that none of the surviving animals in the two treatment arms exhibited CNS pathology attributable to anthrax, all treated animals received a fluoroquinolone in this study.

Similar findings were noted in the combination ciprofloxacin and raxibacumab study in monkeys. No animals in the ciprofloxacin group died during 28 days following exposure. Only one animal in the ciprofloxacin/raxibacumab treatment group died of anthrax disease. None of the surviving animals were sacrificed for microscopic examination.

Human volunteers

No serious, fatal or life-threatening events attributed to raxibacumab were observed in clinical studies. The most common adverse reaction reported in raxibacumab treated subjects were headache, upper

respiratory infections and rash. In study HGS1021-C1064, 6/27 (22%) of volunteers without prior administration of diphenhydramine developed rash and urticaria after infusion of raxibacumab. As a result of this finding, patients are to receive diphenhydramine pretreatment before raxibacumab infusion.

In a safety database of 326 subjects, only events that occur with frequency of 1% or greater could be excluded with 95% confidence. As the safety database consisted only of healthy volunteers, any possible study agent/disease interaction could not be assessed. While CNS AEs were reviewed in healthy volunteers, it appears from the animal models that systemic anthrax disease must be present for CNS pathology to be observed. CNS safety review in humans was therefore limited.

Absence of data in the pediatric age range was noted as a limitation of the raxibacumab safety evaluation.

To address the deficiencies identified during review and AIDAC discussion of the original submission, the sponsor conducted additional studies that are subject of the BLA resubmission dated June 15, 2012.

2.2 *Animal Rule (New Drugs and Biologics)*⁷

Products for the treatment or prevention of human disease are studied in adequate and well-controlled clinical trials that enroll patients with the disease of interest, and these clinical trials, along with other investigations and studies, serve as the basis for approving or licensing the product. However, inhalational anthrax is an example of a human disease that is extremely rare and human studies are not feasible. In situations where human efficacy studies cannot be conducted because it would be unethical to deliberately expose healthy human volunteers to a lethal or permanently disabling toxic biological, chemical, radiological, or nuclear substance, and field trials to study the product's efficacy after an accidental or hostile exposure to the agent have not been feasible, efficacy of new products (e.g., raxibacumab for the treatment of inhalational anthrax) need to be derived from animal models of the disease.

FDA published a Final Rule in 2002 titled “New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible,”⁸ that is also referred to as the “Animal Rule.” As noted above, this rule applies when adequate and well-controlled studies in humans cannot be ethically conducted and field efficacy studies are not feasible. In such circumstances, products intended to reduce or prevent serious or life-threatening conditions may be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals and additional supporting data.

This rule is summarized in the Code of Federal Regulations (CFR), in Title 21 CFR 314.600 (Subpart I) for New Drugs and in Title 21 CFR 601.90 (Subpart H) for Biologic Products. Raxibacumab is a monoclonal antibody, and therefore, the provisions of 21 CFR 601.90 (Subpart H) regulations apply. Excerpts and summaries from the Final Rule and the CFR are provided below and describe the type of evidence and information that need to be provided to the FDA for determination of efficacy.

2.2.1 *Criteria for Submission*

In January 2009, FDA published a draft *Guidance for Industry: Animal Models – Essential Elements to Address Efficacy Under the Animal Rule*,⁹ which provides information on the development of animal

⁷ Final Rule: 67 FR 37988 (May 31, 2002); Regulations: 21 CFR § 601.90-95 (Biologics)

⁸ Final Rule published in the Federal Register, Vol. 67, No. 105, May 31, 2002, pages 37988-37998; Regulations: 21 CFR § 314.600-650 (New Drugs), 21 CFR § 601.90-95 (Biologics)

⁹ <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065014.htm>

models to study efficacy, including the critical characteristics of an animal model that need to be addressed under the Animal Rule. For example, these elements include the characteristics of the agent that causes the disease (in this case *B. anthracis*), the host susceptibility and response to the agent, the natural history of the disease in humans and comparability to animal models, the trigger and timing of intervention, the characteristics of the medical intervention, and study design considerations.

All animal studies subject to this rule must be conducted in accordance with Good Laboratory Practices (GLP) requirements.

Safety evaluation of products is not addressed in the Animal Rule. Products seeking approval under the Animal Rule are expected to be evaluated for safety under preexisting requirements for establishing the safety of new drugs and biological products. Information is expected on the safety of the product, immunogenicity, as well as potential drug interactions (e.g., raxibacumab and antimicrobial). FDA recognizes that some safety data, such as data on possible adverse interactions between the disease and the new product, may not be available.

2.2.2 Criteria for Approval

The Animal Rule states that a biologic (e.g., raxibacumab) can be approved on the basis of adequate and well-controlled animal studies when the results of those animal studies establish that the biologic product is reasonably likely to produce clinical benefit in humans. In assessing the sufficiency of animal data, FDA may take into account other data, including human data, available to the Agency. FDA will rely on the evidence from studies in animals to provide substantial evidence of the effectiveness of these products only when:

- There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
- The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;
- The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and
- The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

Therefore, data from appropriate studies to address each of the above bullet points would need to be provided to support the conclusion that the product is effective.

In addition, approval under this regulation will be subject to three requirements:

1. Postmarketing studies - The applicant must conduct postmarketing studies, such as field studies, to verify and describe the biologic product's clinical benefit and to assess its safety when used as indicated when such studies are feasible and ethical (for example during or following a bioterrorism event).
2. Approval with restrictions to ensure safe use - If FDA concludes that a biologic product shown to be effective under this regulation can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to ensure safe use of the biological product.
3. Information to be provided to patient recipients - For biologic products or specific indications approved under this subpart, applicants must prepare, as part of their proposed labeling, labeling to be provided to patient recipients that includes the following: an explanation that the drug's

approval was based solely on animal efficacy studies, the indication, directions for use, contraindications, adverse reactions, foreseeable risks and benefits, drug interactions, and any other relevant information required by FDA at the time of approval.

3. Microbiology of Anthrax and Raxibacumab Activity

3.1 *Bacillus anthracis*

B. anthracis is a Gram-positive, toxin-producing, encapsulated, spore-forming, facultative anaerobic bacillus. The major virulence factors for *B. anthracis* are the capsule and anthrax toxins. The *B. anthracis* capsule enables the vegetative form of *B. anthracis* to avoid phagocytosis. All three components of anthrax toxin: protective antigen (PA), lethal factor (LF), and edema factor (EF) are present during established infection. PA is present on the surface of spores, and human monoclonal antibody can bind the PA on the surface of spores.¹⁰ PA is the receptor-binding toxin component that allows intracellular entry of LF and EF. EF is a calmodulin-dependent adenylate cyclase that induces edema in various tissues. LF is a zinc metalloprotease that cleaves and inactivates mitogen-activated protein kinase kinases (MAPKKs), key signal transduction molecules required for effective host responses against bacterial pathogens as well as cellular functions.

Monomeric PA secreted as an 83 kDa protein binds one of two cellular receptors, TEM8/ATR or CMG2. PA₈₃ is then cleaved by a host protease, such as furin, to remove an N-terminal 20 kDa fragment. Seven 63 kDa fragments of PA then assemble into a heptamer ring on the cell surface leading to the formation of binding sites for up to three molecules of LF and/or EF. The toxin complex is internalized by endocytosis, a pore is formed in the acidic environment of the endosome, and LF and EF are extruded into, and exert their effects in, the cytosol.¹¹

Lethal toxin (LT), comprised of LF+PA, targets a variety of cell types, including immune cells (macrophages, dendritic cells, neutrophils and lymphocytes), leading to disruption of immune responses, and thereby facilitating infection. LT also has toxic effects on endothelial cells, leading to loss of barrier function, which is thought to be a major factor underlying the pathology induced by LT. Similarly, edema toxin (ET), comprised of EF +PA, has wide-ranging effects through its enzymatic activity that results in increased cellular cyclic AMP, a critical cellular signaling molecule. In addition to mediating edema, ET has immunomodulatory effects and perturbs endocrine function.¹² The secreted *B. anthracis* toxins are thought to be responsible for the morbidity and high mortality rates characteristic of anthrax infection despite appropriate antimicrobial therapy.

3.2 *Raxibacumab*

Raxibacumab is a recombinant, fully human, IgG1 λ monoclonal antibody directed at the PA of *Bacillus anthracis*.

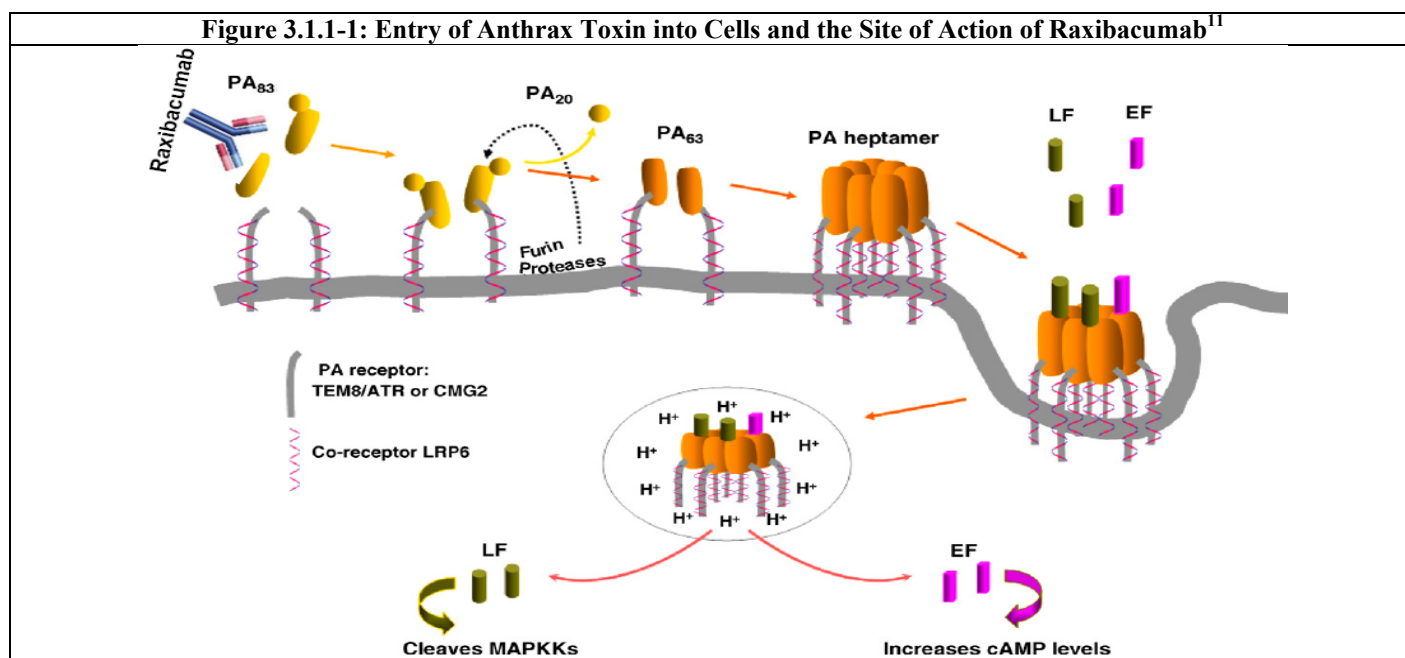
¹⁰ Cote, C, Rossi, CA, Kang, AS, Morrow, PR, Lee, JS, and Welkos, SL. The detection of protective antigen (PA) associated with spores of *Bacillus anthracis* and the effects of anti-PA antibodies on spore germination and macrophage interactions. *Microbial Pathogenesis* (2005) 38: 209-225.

¹¹ Xu, L and Frucht, DM. *Bacillus anthracis*: a multi-faceted role for anthrax lethal toxin in thwarting host immune defenses. *Internat. J. Biochem. Cell Biol* (2007) 39: 20-4.

¹² Moayeri and Leppla, Cellular and systemic effects of anthrax lethal toxin and edema toxin. *Mol Asp of Med*, 2009, in-press, but on-line.

3.1.1 Mechanism of Action

In vitro studies demonstrated that raxibacumab binds PA in supernatants from Sterne, Ames and Vollum strains of *B. anthracis*. The mechanism of action appears to be by neutralization of free PA. Studies show that PA binds the surface of human and murine macrophages, and the epitope on recombinant PA that binds the cell surface receptor appears to be the same as the one binding raxibacumab (Figure 2)¹¹, thereby suggesting that some structural similarity between the antigen recognition site on raxibacumab and the cell surface receptors binding site for PA. Raxibacumab blocks cAMP induction by recombinant ET when PA is preincubated with raxibacumab prior to addition of EF. Similarly, raxibacumab inhibits killing of murine macrophage (J774-A.1) cells when PA is preincubated with raxibacumab prior to addition of LF.



PA₈₃ binds one of two cellular receptors, TEM8/ATR or CMG2, which have been reported to be associated with the LRP6 co-receptor. After binding, PA₈₃ is cleaved by cellular proteases, such as furin, and the small PA₂₀ fragment is released. PA₆₃ then forms a ring-shaped heptameric pre-pore, which can simultaneously bind up to three molecules of LF and/or EF. The toxin/receptor complex is then internalized. The endocytic vesicles are subsequently acidified, initiating a conformational change of the PA heptamer which converts it from the pre-pore into a mature pore that allows entry of EF and/or LF into the cell cytoplasm. LF is a protease targeting specific MAPKKs. EF is an adenylate cyclase that increases cAMP formation in cells. Raxibacumab blocks the ability of PA to bind its cellular receptors.

3.1.2 Activity *in vivo*

Rat Toxin Model

The effect of raxibacumab was evaluated in a toxin rat model. In F344 rats, intravenous (IV) administration of raxibacumab at a concentration of ten-fold the concentration of recombinant PA (rPA), administered up to three weeks prior to challenge with rLT (rLF + rPA), resulted in 100% survival for 24 hours. Isotype-antibody-treated control rats died within 90 minutes of rLT administration. Survival beyond 24 hours was not measured.

Rabbit: Pre-exposure and Post-exposure Prophylaxis.

The effect of raxibacumab in pre-exposure and post-exposure prophylaxis was evaluated in NZW rabbits in GLP studies.

The applicant evaluated the effect of a single IV dose of 40 mg/kg raxibacumab administered at 0, 12, 24, or 36 hours post-exposure on survival in rabbits. Raxibacumab, at a single dose of 40 mg/kg, was effective in improving survival (100%) and clearing bacteremia when administered at the time of exposure or 12 hours post exposure. However, if the treatment was initiated at 24 or 36 hours post exposure, the survival decreased to 50% and 42%, respectively.

In another experiment, the efficacy of single doses (5, 10, 20, or 40 mg/kg) of raxibacumab administered IV at 24 or 36 hours post-exposure was measured. A single IV dose of 20 mg/kg raxibacumab administered at 24 hours post exposure was more effective in improving survival up to 14 days, when compared to the placebo treated rabbits ($p=0.0373$). Survival in the other treatment groups, including the highest dose tested (40 mg/kg), was not different from the placebo group. Initiation of treatment at 36 hours post infection was not effective in improving survival.

In the 3rd experiment, raxibacumab was administered at different doses (1, 5, 10, or 20 mg/kg) subcutaneously (SC) two days prior to exposure (aerosol challenge) or intravenously (40 mg/kg) within one hour of challenge with *B. anthracis* spores. Raxibacumab doses of 5, 10, and 20 mg/kg administered subcutaneously (SC) two days prior to spore challenge increased survival and reduced bacteremia. All rabbits receiving 40 mg/kg raxibacumab by the IV route within one hour of infection survived until 14 days post exposure.

Cynomolgus Macaques: Pre-exposure prophylaxis

The effect of a single SC dose (10, 20, or 40 mg/kg) of raxibacumab was also evaluated in a pre-exposure (2 days) prophylaxis GLP study in cynomolgus macaques. Survivors were followed for an additional 11 months and were re-challenged. Raxibacumab improved survival at all dose levels evaluated with 60%, 70% and 90% survival at 10, 20 and 40 mg/kg doses, respectively.

At 11 months after the original exposure, twenty one surviving monkeys were re-challenged with *B. anthracis* spore aerosol at 100xLD₅₀ and survived to 28 days after re-challenge. All six control animals died by Day 6 post exposure. All surviving monkeys had neutralizing antibody titers following the initial exposure to *B. anthracis* spores, and the titers increased following the second exposure to *B. anthracis*.

4. Anthrax Disease – Natural History in Animal Models and Comparison to Human Disease

In order to be able to develop a product such as raxibacumab for the treatment of inhalational anthrax, it was important to identify and characterize animal models of inhalational anthrax to be used to evaluate product's efficacy. After consideration of rodent and larger animal species, the natural history of anthrax in cynomolgus macaques and New Zealand White rabbits was evaluated, and these animal species were subsequently used for the evaluation of raxibacumab efficacy in randomized comparative studies.

Table 4.4-1 represents the summary of nonclinical and clinical data from the published literature as well as the sponsor collected nonclinical data from natural history and pivotal/comparison animal studies, and

describes the similarities and differences of anthrax infection, host susceptibility and response, pathophysiology, triggers for intervention, and measure of outcome between human disease and the monkey and rabbit models of disease.

Table 4.4-1: Interspecies Comparison, Inhalational Anthrax (FDA)

DATA ELEMENTS	Animal Model		Human
	New Zealand White rabbit (NZW)	Nonhuman primate (NHP, cynomolgus macaque)	
A. Characteristics of the CBRN Agent that Influence the Disease or Condition			
1. The challenge agent	Ames strain <i>B. anthracis</i> spores	Ames strain <i>B. anthracis</i> spores	Many strains, but Ames strain used in 2001 bioterrorism attack
2. Pathogenic determinants	Antiphagocytic capsule, tripartite exotoxin	Antiphagocytic capsule, tripartite exotoxin	Antiphagocytic capsule, tripartite exotoxin
3. Route of exposure	Aerosol challenge with 200x LD ₅₀ of <i>B. anthracis</i>	Aerosol challenge with 200x the LD ₅₀ of <i>B. anthracis</i>	Inhalation
4. Quantification of exposure	200x LD ₅₀ LD ₅₀ = 1.1x10 ⁵ cfu	200x LD ₅₀ LD ₅₀ = 0.6-1.1 x10 ⁵ cfu	Infectious dose 2-55x10 ⁴ cfu estimated from NHP studies and contaminated mills studies
B. Host Susceptibility and Response to Etiologic Agent	Susceptible ¹	Susceptible	Moderately susceptible
C. Natural History of Disease: Pathophysiologic Comparability			
1. Time to onset of disease/condition	16-53 hours postexposure to positive PA/fever. More rapid than humans ²	28-58 hours postexposure to positive PA	Incubation period mostly < 7 days; longest up to 2 months
2. Time course of progression of disease/condition	More rapid than humans. Succumb to disease 2-9 (median 3) days postexposure	Similar to that seen in humans but dependent on the dose used for the challenge. ^{4,5} At 200x LD ₅₀ death 3-9 (median 4) days.	Prodromal phase 1-6 days; fulminant phase less than 24 hours ⁷ Overall mortality of 85% with a mean time from symptom onset to death of 4.8 days ⁷ In 2001 attacks, symptoms began 4-6 days. ⁸ In Sverdlovsk incident, death in 1-4 days. ⁹
3. Manifestations (signs and symptoms)	Lethargy, not eating, rapid respirations, respiratory distress, seizures and/or moribund shortly prior to death	Few clinical signs until 1-2 hours prior to death, then progressively less responsive to external stimuli. Lethargy, tachycardia, and fever, although abnormal signs varied and presented shortly before death. ⁶	Flu-like symptoms for several days followed by severe respiratory collapse
4. Pathology	Mediastinitis, pneumonia, hemorrhagic meningitis 30%	Mediastinitis, pneumonia, hemorrhagic meningitis 30%	Hemorrhagic mediastinitis, meningitis 50%
D. Trigger for Intervention	Positive PA level or temperature elevation	Positive PA level	Variable, circumstance specific – medical history, epidemiologic history, signs and symptoms

DATA ELEMENTS	Animal Model		Human
	New Zealand White rabbit (NZW)	Nonhuman primate (NHP, cynomolgus macaque)	
E. Characterization of the Medical Intervention			
1. Product class	Raxibacumab, human monoclonal antibody		
2. Mechanism of action	Human monoclonal antibody which binds the protective antigen (PA) of <i>B. anthracis</i> and prevents the binding of PA with its receptors		
4. Activity in disease/condition of similar pathophysiology	N/A		
5. PK in unaffected animals/humans	Raxibacumab PK in infected and healthy NZW rabbits was similar		
6. PK/PD in affected animals/humans	Healthy adult humans achieve similar to or greater exposure to raxibacumab at 40 mg/kg single IV dose compared to animals receiving the same dose: <ul style="list-style-type: none">• Mean raxibacumab Cmax in humans was similar to or greater than mean Cmax values in monkeys and rabbits.• Mean raxibacumab AUC in humans following a single 40 mg/kg IV dose was 2.4 and 4.6-fold that of the mean AUC values in monkeys and rabbits, respectively.• T_{1/2} raxibacumab was longer in humans compared to mean half-lives observed in monkeys and rabbits (20.6 ± 6.5 days versus 10.1 ± 2.4 days and 4.1 ± 0.85 days, respectively).		
7. PK interactions with medical products likely to be used concomitantly	No PK interaction with levofloxacin	No PK interaction with ciprofloxacin	No PK interaction with ciprofloxacin
8. Synergy or antagonism of medical products likely to be used in combination	No interaction with levofloxacin	No interaction with ciprofloxacin	
F. Design Considerations for Animal Efficacy Studies	GLP, randomized, double-blind, placebo controlled study with mortality endpoint		
1. Endpoints	Survival	Survival	Survival
2. Timing of intervention	Based on detection of positive PA or 2 consecutive temperature elevations, confirmed by culture	Based on detection of positive PA level, confirmed by culture	Variable upon suspicion, time of diagnosis, or general condition -- empiric
3. Route of administration	IV	IV	IV
4. Dosing regimen	Single dose raxibacumab 40 mg/kg IV	Single dose raxibacumab 40 mg/kg IV	A 40 mg/kg single IV dose resulted in an exposure similar or exceeding those found efficacious in animals
HUMAN SAFETY INFORMATION			323 human subjects exposed to single and repeat dose raxibacumab

1. Phipps AJ, Premanandan C, Barnewall, RE, Lairmore, MD. Rabbit and nonhuman primate models of toxin-targeting human anthrax vaccines. *Microbiol Mol Biol Rev.* 2004 Dec;68(4):617-29.
2. Zaucha GM, Pitt LM, Estep J, Ivins BE, Friedlander AM. The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. *Arch Pathol Lab Med* 1998; 122:982-92.
3. Fritz DL, Jaax NK, Lawrence WB, Davis KJ, Pitt MLM, Ezzell JW, Friedlander AM. Pathology of experimental inhalation anthrax in the Rhesus monkey. *Lab. Invest.* 1995; 73:691-702.

4. Vasconcelos D, Barnwell R, Babin M, Hunt R, Estep J, Nielsen C, Carnes, Carney J. Pathology of inhalation anthrax in *Cynomolgus* monkeys (*Macaca fascicularis*). *Lab. Invest.* 2003;83:1201-1209.
5. Twenhafel NA, Leffel E, Pitt MLM. Pathology of inhalational anthrax infection in the African Green monkey. *Vet. Pathol.* 2007; 44:716-721.
6. Rossi C, Ulrich M, Norris S, Reed DS, Pitt LM, and Leffel EK. *Infect Immun.* 2008; 76:5790-5801.
7. Holty JEC, Bravata DM, Liu H, Olshen RA, McDonald KM, Owens DK. Systematic review: a century of inhalational anthrax cases from 1900 to 2005. *Ann. Intern. Med.* 2006;144:270-280.
8. Bell, DM, Kozarsky, PE, Stephens, DS. Clinical Issues in the Prophylaxis, Diagnosis, and Treatment of Anthrax. *Emerg Infect Dis* 2002. 8 (2): 222-5
9. Abramova, FA, Lev M. Grinberg LM, Yampolskaya, OV, And. Walker, DH. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979 *Proc. Natl. Acad. Sci. USA* 1993; 90:2291-4.

5. New Animal Efficacy Studies

This section describes two new animal studies included in the resubmission of the BLA for raxibacumab. These studies were conducted to support the added benefit (over antibacterials) of raxibacumab treatment and to provide additional information on the CNS toxicity findings seen in the original BLA submission.

5.1 CNS Toxicity Study in Rabbits

Study 1103-G923704: “Evaluation of Raxibacumab as a Therapeutic Treatment Against Inhalational Anthrax in the New Zealand White Rabbit Model”

Objectives:

- To assess terminal pathology in select organs, particularly in the CNS, in both surviving and non-surviving rabbits
- To evaluate the efficacy of raxibacumab as a monotherapy against lethality in rabbits with inhalational anthrax

5.1.1 Study Design

This was a parallel-group, blinded, randomized, placebo-controlled GLP raxibacumab monotherapy study to evaluate pathology in the brain and other select organs after raxibacumab treatment in the rabbit inhalational anthrax model. Survival, bacteremia, raxibacumab and protective antigen (PA) kinetics, and brain immunohistochemistry for raxibacumab were also examined.

Methods:

The study was conducted at Battelle Biomedical Research Center, West Jefferson, OH. Forty-eight (50% male, 50% female), 7-month-old New Zealand White rabbits with surgically implanted vascular access ports (VAP), weighing between 2-5 kg were randomized into two treatment groups (placebo control and 40 mg/kg raxibacumab) and then further randomized into 1 of 2 aerosol challenge days and challenge order (See Table 5.1-1 below).

Table 5.1-1: Treatment Groups

Groups	N	Dose	Route/Frequency	Treatment Point
Group 1 (Placebo)	24	Placebo ¹	IV / single dose	Individual treatment times based on serum PA levels ²
Group 2	24	40 mg/kg	IV / single dose	

¹ Raxibacumab buffer, administered at an equivalent volume in mL/kg to Group 2

² Each rabbit received raxibacumab or placebo immediately upon its 1st positive PA result in the qualitative ECL assay.

(Adapted from HGS² Study Report, p. 14)

On their designated challenge day, each animal was challenged with *B. anthracis* (Ames strain) aerosol at a target 200 LD₅₀ [2.1x10⁷ spores] dose. Aerosol concentrations of *B. anthracis* were quantified by determination of colony forming units (cfu) in effluent streams. Test article or control buffer was administered in a single intravenous bolus of 40 mg/kg raxibacumab or buffer (placebo) upon detection of positive qualitative PA results (≈ 16-48 hr post-challenge) using a chemiluminescence (ECL) assay. Surviving animals were euthanized on Day 28 post challenge. The study director, technical staff and study pathologists were blinded to treatment.

Animal observations and temperature monitoring occurred every 6 hours between 18 and 168 hours post-challenge, and twice daily on all other study days (beginning 3 days prior to challenge). Death or euthanasia was recorded at the time observed. Blood samples were collected at several study time points and assayed for bacteremia, plasma PA, plasma raxibacumab concentrations, immunogenicity (anti-raxibacumab antibodies), and toxin neutralizing antibodies (TNA). No raxibacumab PK or PA kinetic modeling was performed. Cerebrospinal fluid was collected at necropsy and assessed for *B. anthracis* culture, quantitative PA and raxibacumab concentrations. Complete gross necropsies were conducted on all rabbits that succumbed to disease and those survived to study termination. Brain, lungs, spleen, liver, kidney, and mediastinal/bronchial lymph nodes were collected and evaluated for gross and microscopic findings. A brain sample was collected for culture at necropsy. All tissues were then fixed by immersion into formalin; brain and spleens were post-fixed in 70% ethanol and dissected into the following 4 sections:

- (A) cerebral cortex (frontal parietal), corpus callosum, and basal ganglia;
- (B) hippocampus, cerebral cortex (temporal-parietal), thalamus/hypothalamus;
- (C) cerebellum including vermis and hemispheres, and medulla;
- (D) Midbrain (colliculi).

Prepared slides from the 4 brain sections were processed with the following stains and by immunohistochemistry (IHC):

- (A) Bielschowsky stain for neurodegeneration and neurofibrillary plaques;
- (B) Fluoro-Jade C stain for acute neuronal degeneration;
- (C) IHC for Glial Fibrillary Acid Protein (GFAP) to detect chronic or healed damage;
- (D) IHC for Raxibacumab;
- (E) IHC for Rabbit IgG.

The study pathologist was blinded to the treatment until after the slides were read.

5.1.2 Results

Anthrax Exposure

The two treatment groups were comparable with respect to sex, weight, and age at randomization. Anthrax spore challenge was slightly greater in the raxibacumab treatment group; however, the average dose in the placebo group was confirmed to be lethal as all placebo-treated animals succumbed to disease following challenge. The anthrax spore challenge by treatment group is shown in Table 5.1-2 below.

Table 5.1-2: Average Challenge Doses (LD₅₀ equivalent)

Challenge Day	Average Challenge Dose (SD)
A	142 (± 45)
B	149 (± 36)
Group	Average Challenge Dose (SD)
1 (40 mg/kg Raxibacumab)	154 (± 41)
2 (Buffer - placebo)	138 (± 39)

(Table 3 on page 21 in the Battelle Study Report)

Timing of Treatment

Treatment with raxibacumab was triggered in each animal individually by the first qualitative positive plasma PA result (ECL). Animals in the placebo and raxibacumab treatment groups were treated at a mean time of 32.3 and 28.6 hours after challenge, respectively. There were no significant differences in the average time of onset toxemia or bacteremia or the number of toxemic and bacteremic animals between treatment groups at time of intervention.

Mortality and Survival

The mortality rates for both study groups are shown in Table 5.1-3 below. None of the placebo animals survived, whereas 46% (11/24) of the raxibacumab treated animals survived to Day 28. Most of the survivors (11/13) in the raxibacumab-treated group were female in this study.

Table 5.1-3: Primary Efficacy Analysis, Survival at Day 28 postexposure (FDA analysis)

	Placebo	Raxibacumab	Difference (Raxi – Placebo) (95% CI)(%)	P-value
ITT animals	0/24 (0.0%)	11/24 (45.8%)	45.83 (25.3, 67.2)	0.0002
Toxemic animals at or before treatment	0/24 (0.0%)	10/23 (43.5%)	43.48 (22.9, 65.5)	0.0002
Bacteremic animals at or before treatment	0/22 (0.0%)	10/23 (43.5%)	43.48 (22.5, 65.5)	0.0006
Animals excluding non-anthrax death	0/24 (0.0%)	11/23 (47.8%)	47.8 (26.6, 69.4)	<0.0001

*P-values are based on two-sided Fisher's exact test for comparisons between the treatment and placebo

The sponsor reported a greater time of survival in animals both toxemic/bacteremic at treatment initiation with a median time to death (TTD) of 3.3 days for placebo versus 8.0 days (p-values ranging from 0.0002 to 0.0007) for the raxibacumab group (see Figure 5.1-1 below).

Figure 5.1-1: Survival Time During 28-day Study Period in Rabbits Toxemic and/or Bacteremic at Treatment Initiation

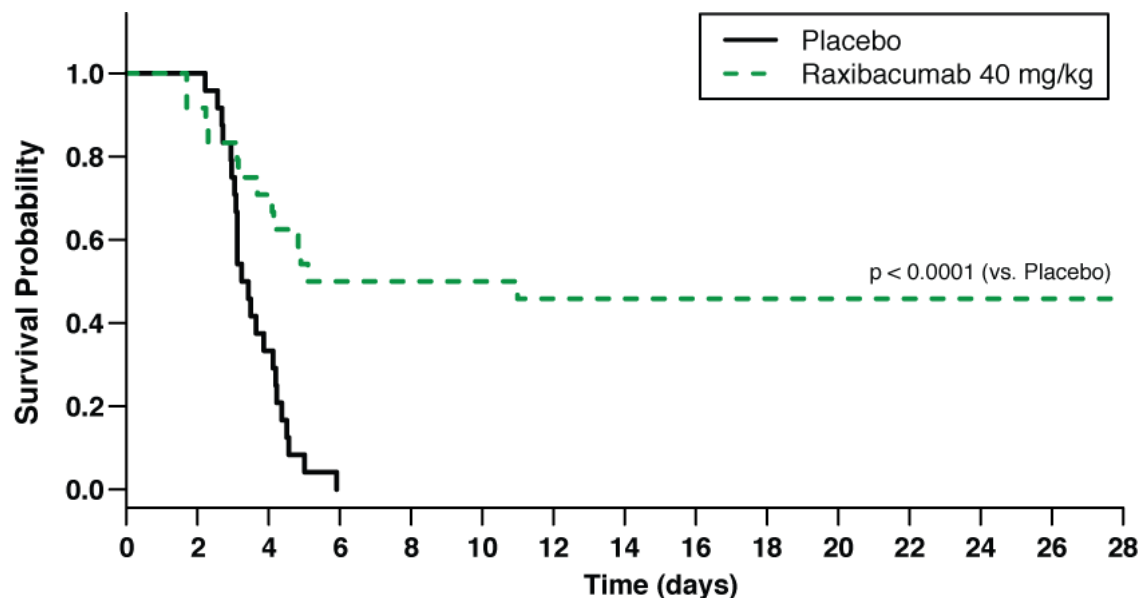
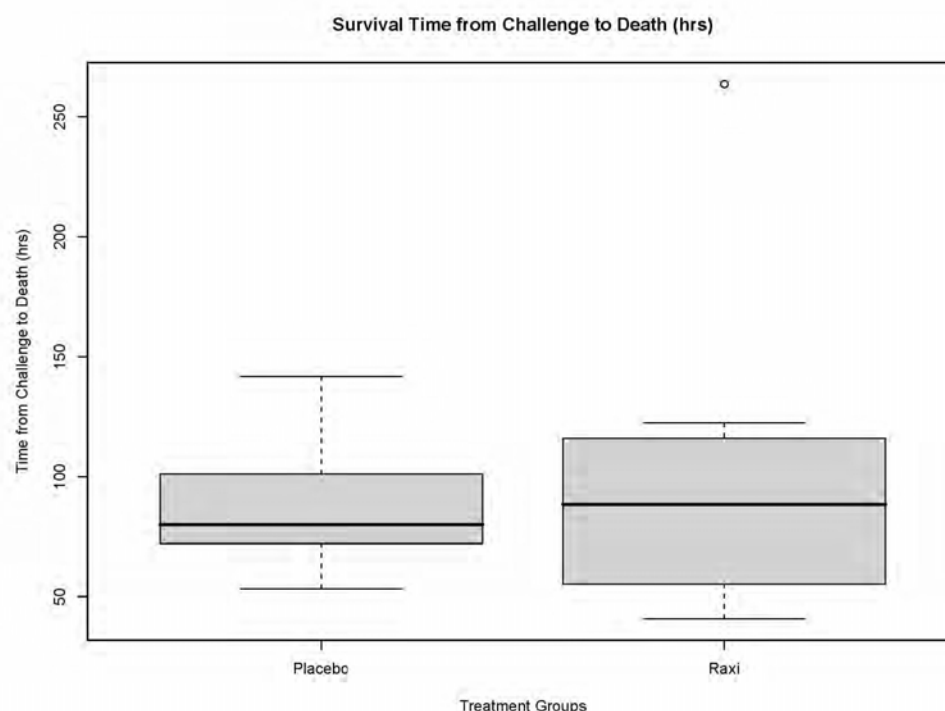


Figure 7-3 from HGS report Page 35.

P-value obtained from a log-rank test comparing survival time between placebo and raxibacumab-treated groups.

Additional analysis on survival time observed in non-surviving rabbits that died prior to Day 28 showed no significant difference in mean TTD post-challenge in raxibacumab and placebo treated animals (mean 3.99 versus 3.59 days, respectively), with an approximate difference of only 9.7 hours (Note: The TTD listed is an estimate of the TTD post-challenge since a majority of dead animals in both groups were found dead in their cages). Figure 5.1-2 shows the distribution of TTD post-challenge for animals that died in the placebo and raxibacumab groups. The raxibacumab animal (#L35568) that died at 263.7 hours after anthrax challenge did not have extravascular bacteria and the cause of death was deemed unlikely to be anthrax related [note: the study pathologist indicated possible renal infection of a parasitic fungi (*Encephalitozoon cuniculi*)].

Figure 5.1-2: Survival Time from Challenge to Death in Non-Surviving Raxibacumab and Placebo Treated Animals (FDA analysis)



Blood, CSF, and Brain Tissue Culture Levels of *B. anthracis*

Ninety-four percent (45/48) of rabbits dosed with anthrax were bacteremic prior to treatment. Nearly all (34/37) placebo and raxibacumab treated animals that died prior to the 7 day post-challenge time point showed positive bacteremia results.

CSF was successfully collected from 65% (24/37) of the rabbits that died on study and all (11/11) rabbits that survived to 28 days post-challenge. All (19/19) of the placebo-treated animals from which a CSF sample could be collected had a positive bacteria culture; eighty percent (4/5) of the non-surviving raxibacumab treated animals from which a CSF sample could be collected were positive for bacteria. Similarly, 100% (24/24) of the rabbits in the placebo group and 92% (12/13) of the non-surviving raxibacumab treated animals had positive brain tissue cultures. Bacterial infiltration into the CNS (both in the CSF and nervous tissue) was confirmed in nearly all non-surviving animals in the placebo and raxibacumab groups.

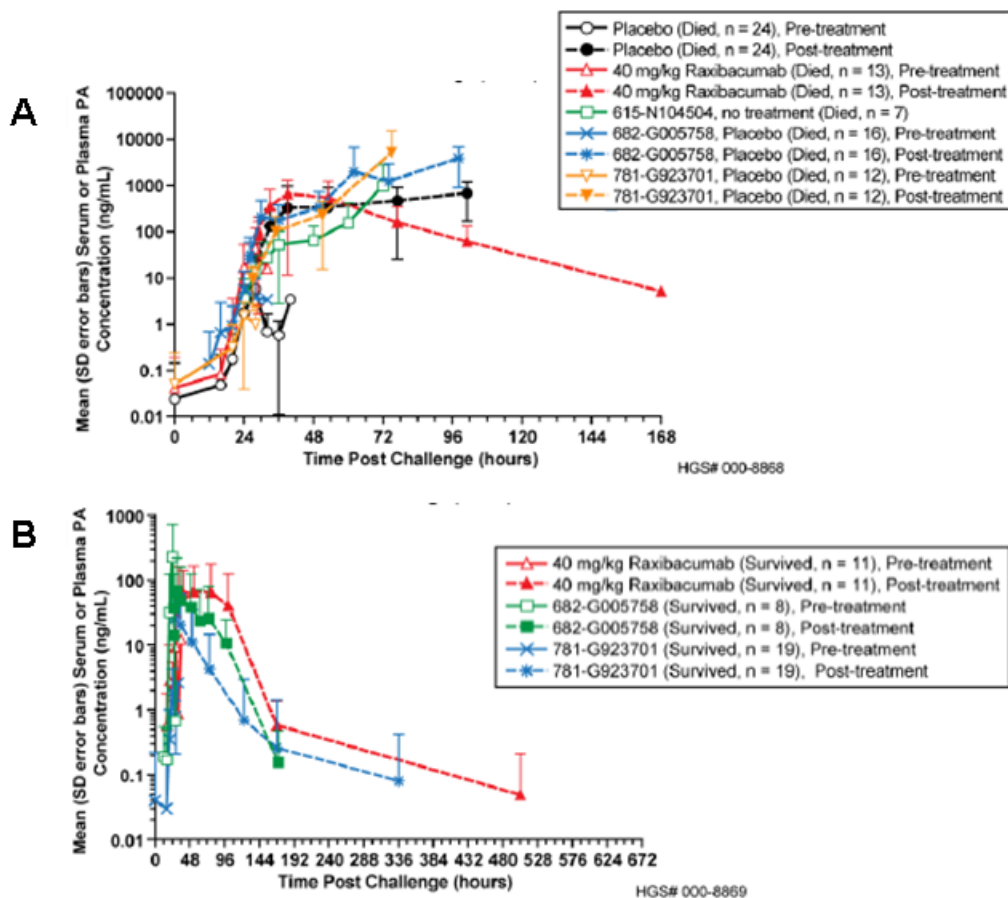
All (11/11) surviving rabbits were negative for bacteremia 7 days post-challenge and showed no bacteria in the CSF or brain tissue cultures by day 28.

Quantitative Plasma and Cerebrospinal Fluid (CSF) PA Levels

The sponsor's mean plasma PA concentration-time profiles for the dead and surviving animals treated with placebo or 40 mg/kg raxibacumab are shown below in Figure 5.1-2 (data from the original raxibacumab monotherapy (682) and raxibacumab + levofloxacin combination therapy (781) rabbit studies included in graph). Non-surviving animals in both groups showed a similar PA profile, which appeared different from surviving animals. Up to the time of treatment, mean plasma PA concentrations were similar to the placebo group. After treatment, the few non-surviving raxibacumab treated rabbits

that survived beyond the mean TTD (2.89 days post-treatment) showed a relative decrease in plasma PA concentrations (Figure 5.1-3A). However, in surviving animals in the raxibacumab group, mean plasma PA concentration-time profiles showed an approximate 10-fold decrease of the mean peak plasma PA concentration compared to placebo. The decrease in plasma PA concentrations could be a result of raxibacumab-PA complex formation and decreased detection in the assay, or from bacteremia clearance or inhibition of spore germination¹³.

Figure 5.1-3: Plasma PA Concentration-Time Profiles in non-surviving (A) and surviving (B) rabbits administered a single IV bolus dose placebo or 40 mg/kg raxibacumab after detection of PA toxemia



(Figures 6-4 and 6-5 on page N-24 of the Battelle study report)

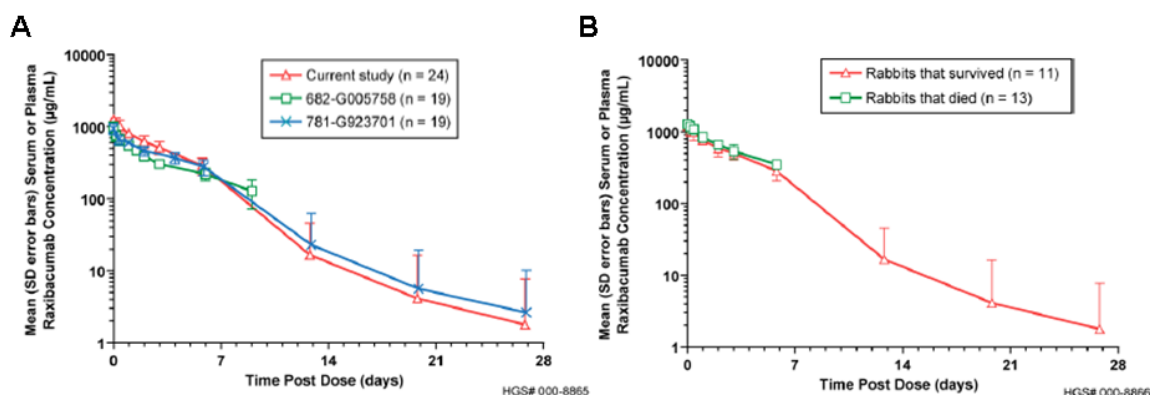
None (0/11) of the surviving raxibacumab animals showed measurable PA levels in the CSF. In animals that died, CSF PA levels were highly variable in both treatment groups, ranging from not detectable to > 966 ng/mL (0% to 37% of concurrent plasma PA concentrations in the placebo group). The high variability of PA levels in the subset of animals from which CSF could actually be collected made comparisons of mean PA levels difficult to interpret.

¹³ Welkos, S., Little, S., Friedlander, A., Fritz, D., and Fellows, P. (2001). The role of antibodies to *Bacillus anthracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. *Microbiology*. 147, 1677-1685.

Plasma and CSF Raxibacumab Levels

The mean observed plasma raxibacumab concentration-time profile for the 40 mg/kg raxibacumab dose group is similar to the drug profile obtained in other studies, included in Figure 5.1-4A for comparison below. Also, plasma raxibacumab concentrations in dead and surviving raxibacumab treated rabbits were similar, indicating survival was not attributed to differences in raxibacumab exposure (Figure 5.1-4B).

Figure 5.1-4: Mean plasma raxibacumab concentration-time profiles in rabbits administered a single IV bolus 40 mg/kg raxibacumab after PA detection compared with results from other studies (A); mean raxibacumab plasma levels in dead and surviving rabbits administered 40 mg/kg raxibacumab after detection of PA (B).



(Figures 6.1 and 6-2on pages N-18 and N-19 of the Battelle study report)

Overall, raxibacumab appears to minimally distribute into the CSF of treated animals. Similar to the CSF PA results, raxibacumab concentrations in the CSF measured in (4/13) treated rabbits that died (limited by an inability to collect a sufficient CSF sample) showed variable results ranging from < 1 µg/mL to 545 µg/mL (0.2-88% of the concurrent plasma raxibacumab concentration). Raxibacumab was detected in the CSF of a few surviving animals at very low concentrations (range: 0 to 0.331 µg/mL). However, highly variable raxibacumab CSF levels in the subset of animals from which CSF could actually be collected made comparisons of mean raxibacumab levels difficult to interpret.

Immunogenicity (Anti-Raxibacumab Antibodies) and Toxin Neutralizing Antibody Titers

Immunogenicity and toxin neutralizing antibody (TNA) titer results were available at pre-dose for all animals and for Day 28 in survivors only; no post-dose titer data is available for animals that died before Day 28 due to assay interference with plasma raxibacumab.

At pre-dose, (3/24) rabbits in the placebo group were positive for anti-raxibacumab antibodies (Ab) with post-dose outcome unknown for all animals in this group (all animals died). Of the surviving animals in the raxibacumab treatment group, (3/11) were negative for anti-raxibacumab Ab at baseline and post-dose; (7/11) rabbits were negative at baseline and positive for anti-raxibacumab Ab post-dose; and (1/11) rabbits was positive for anti-raxibacumab AB at baseline and post-dose. All (13/13) non-survivors in the raxibacumab group were negative for anti-raxibacumab antibodies at pre-dose with the post-dose outcome unknown. Although 8/11 surviving rabbits were anti-raxibacumab antibody positive, the diminished raxibacumab exposure generally observed after Days 6-7 post-treatment did not appear to affect survival suggesting one or more of the following: 1) the anti-raxibacumab antibodies were not neutralizing; 2) raxibacumab was present but the assay was unable to measure the raxibacumab-Ab complexes; 3)

adequate immunologic response to bacteria and toxins after 6-7 days post-challenge can prevent further deaths in this group.

Raxibacumab did not interfere with development of endogenous TNA; high TNA titers ($\mu \approx 2175$) detected only in surviving rabbits 28-days post-treatment were generated in the presence of high plasma raxibacumab levels after treatment.

5.1.3 Necropsy and Histopathology

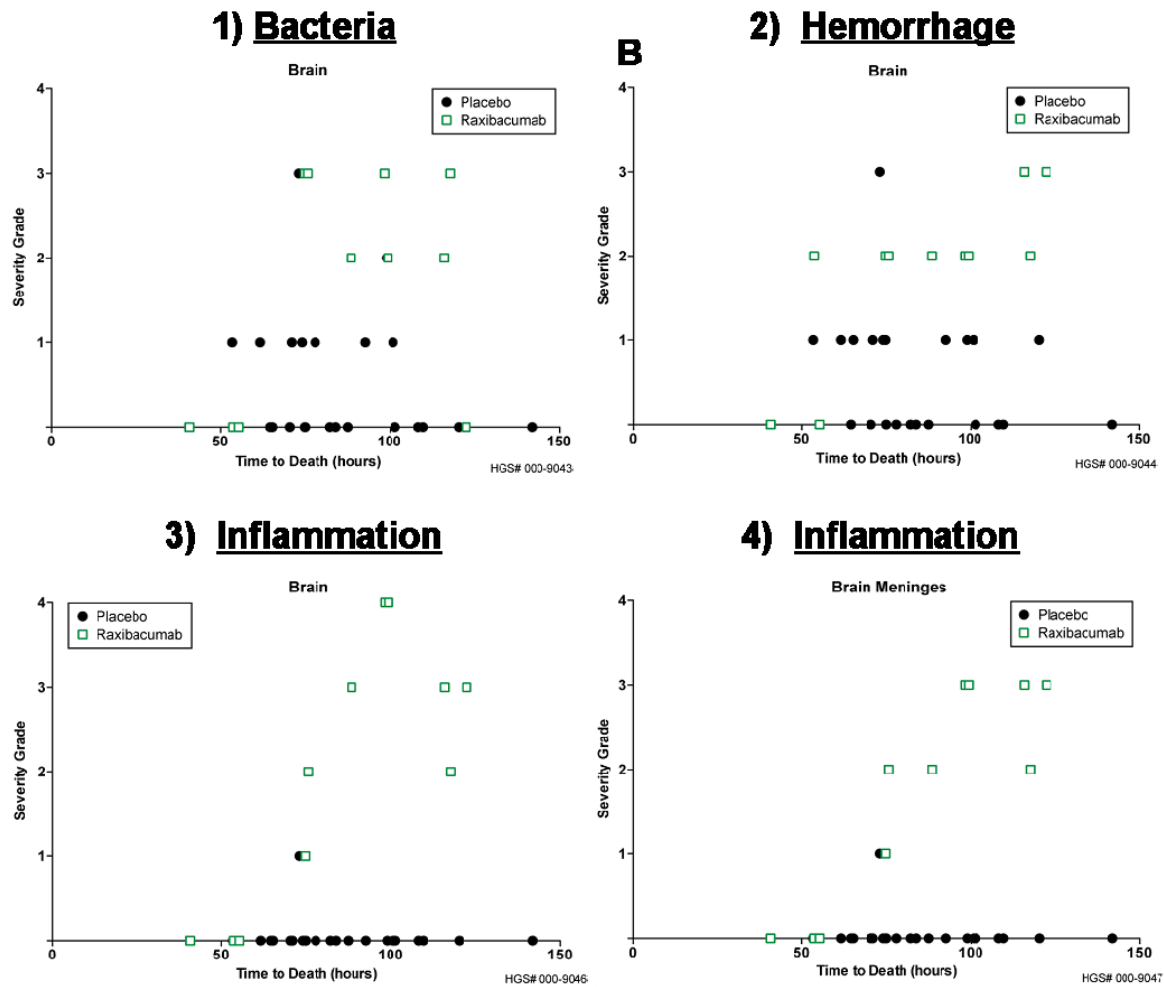
Gross lesions in rabbits found dead or euthanized moribund included discoloration or foci in the appendix and the brain (hemorrhage and inflammation), enlargement of bronchial and mediastinal lymph nodes (edema, fibrin exudation, hemorrhage), and fluid (effusion) in the body cavities and thymus. These lesions consistent with anthrax infection¹⁴ were found primarily in rabbits that died or became moribund during the study, correlated histologically with acute suppurative inflammation, necrosis, hemorrhage, edema, vasculitis, and the presence of large-rod shaped bacteria in the appendix, brain, kidney, liver, lung, bronchial and mediastinal lymph nodes, spleen, and thymus. In surviving animals in the raxibacumab group euthanized on Day 28, no gross lesions (attributed to infection or treatment) were detected. However, several minor lesions were noted, including continued inflammation, increased macrophage counts, lymph node and splenic hyperplasia, and chronic inflammation in the lungs suggesting recovery from a septicemic event.

CNS histopathology

In non-surviving animals in the raxibacumab treatment group, brain lesions (bacteremia, inflammation, hemorrhage, and/or necrosis) of greater incidence and severity were observed as compared to non-surviving animals in the placebo group (See Figure 5.1-5, Table 5.1-4 below). Also, non-surviving animals within the raxibacumab treatment group that lived the longest appeared to show the most severe lesions. The lesions appeared widespread throughout the brain; no specific brain region was preferentially affected. Bacterial load in the brain was far more severe in the raxibacumab group compared to control. Inflammation commonly found throughout the brain, especially in the cerebral cortex, hippocampus, ventricular system, cerebellum, and meninges of raxibacumab-treated animals was nearly non-existent in the placebo animals. Similarly, necrosis was more common in the non-surviving raxibacumab-treated animals, specifically in the cerebellum, cerebral cortex, and parenchymal tissue, and was absent in the placebo group. Fluoro-Jade C staining for necrosis/neurodegeneration was only present in the raxibacumab-treated rabbits. No significant glial fibrillary acidic protein (GFAP) positive staining of neural tissue was detected. As in raxibacumab-treated animals, brain hemorrhage was detected in placebo animals at many sites, but with mostly diminished incidence and severity compared to the raxibacumab-treated group; however, meningeal hemorrhage was similar in incidence and severity in both groups. Cerebrovascular necrosis/vasculitis observed in the brains of raxibacumab-treated animals were generally absent in placebo animals. No brain lesions were observed in surviving animals.

¹⁴ Zaucha, G.M., Pitt, L.M., Estep, J., Ivins, B.E., and Friedlander, A.M. (1998). The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous inoculation. *Arch Pathol Lab Med.* 122(11): 982-992

Figure 5.1-5: Severity of Brain 1) Bacteria, 2) Hemorrhage, 3) Inflammation, and 4) Inflammation (Meninges) by Time of Death in Non-Survivors



Adapted from HGS study report Figures 13-1, 13-6, 13-11, and 13-12 (pp 133-144)

Table 5.1-4: CNS Histopathology Findings in Non-Survivors

Group	Raxibacumab Incidence (Mean Severity)	Placebo Incidence (Mean Severity)
Number In Group	24	24
Mortality	54%	100%
# dead	13	24
Bacteria, Extravascular	7 (1.4)	8 (0.5)
Hemorrhage(s)		
Hemorrhage(s), Cerebellum	8 (1.1)	2 (0.1)
Hemorrhage(s), Cerebral Cortex, Fronto-Parietal	6 (0.5)	3 (0.2)
Hemorrhage(s), Cerebral Cortex, Temporo-Parietal	5 (0.5)	3 (0.1)
Hemorrhage(s), Hippocampus	4 (0.5)	0 (0)
Hemorrhage(s), Meninges	9 (1.0)	9 (0.5)
Hemorrhage(s), Midbrain	4 (0.5)	4 (0.2)
Hemorrhage(s), Thalamus	6 (0.5)	3 (0.1)
Inflammation		
Inflammation, Cerebellum	3 (0.4)	0 (0)
Inflammation, Cerebral Cortex, Fronto-Parietal	6 (0.7)	0 (0)
Inflammation, Cerebral Cortex, Temporo-Parietal	6 (0.8)	0 (0)
Inflammation, Heterophils	8 (1.7)	1 (0)
Inflammation, Hippocampus	4 (0.4)	0 (0)
Inflammation, Meninges	8 (1.5)	1 (0)
Inflammation, Ventricular System	4 (0.5)	0 (0)
Necrosis		0 (0)
Necrosis, Cerebellum	3 (0.3)	0 (0)
Necrosis, Cerebral Cortex, Fronto-Parietal	4 (0.4)	0 (0)
Necrosis, Cerebral Cortex, Temporo-Parietal	5 (0.5)	0 (0)
Necrosis, Parenchyma	6 (0.8)	0 (0)
Vasculitis/Cerebrovascular Necrosis	6 (0.7)	1 (0)
Acute Neurodegeneration, FluoroJade-C	5 (na)	0 (na)
Raxibacumab (=) staining, CNS tissue	12 (na)	0 (na)

Severity is defined as 1=minimal, 2=mild, 3=moderate, 4=severe

na: Severity not applicable, severity grade not provided.

(Adapted from Table 8-6 on page 44-45 of the HGS study report)

Raxibacumab immunohistochemistry of the brain

A strong correlation between microscopic brain lesions and frequency, location, and intensity of raxibacumab and endogenous rabbit IgG staining of the brain was observed in non-surviving raxibacumab-treated animals. An overlapping pattern of endogenous IgG and raxibacumab staining of neurons and neuropil was often observed in regions rich with bacteria, inflammation, and/or necrosis. Infection, circulating bacterial toxins, and/or inflammation (including sepsis) has been shown to enhance blood brain barrier (BBB) permeability¹⁵. Based on these results, it appears that raxibacumab and endogenous IgG, both with a limited capacity to cross an intact BBB, may have leaked non-specifically across the damaged blood brain barrier during bacterial meningitis in the raxibacumab group. Only one placebo animal (#L35576) with severe meningitis also stained positive for IgG in the brain suggesting compromise of the blood brain barrier sufficient for IgG and raxibacumab leakage did not generally occur in the placebo group. Surviving animals also may have experienced an altered BBB early in the progression of the disease, as greater endogenous IgG positive staining of vascular endothelium, meninges, and neuropil was observed in raxibacumab-treated survivors compared to unchallenged controls. However, no raxibacumab staining of brain tissue was observed in surviving animals at Day 28.

¹⁵ De Vries, H.E., Kuiper, J., De Boer, A.G., Van Berkel, T.J.C., Breimer, D.D. (1997). The blood brain barrier in neuroinflammatory diseases. *Pharm. Rev.* **49**(2); 143-155.

In contrast to the greater pathologic changes in the brain, raxibacumab was effective in reducing histopathology in all other organs examined, including the appendix, liver, lung, lymph nodes, kidney and spleen in both non-surviving and surviving animals (see Table 5.1-5 and 5.1-6 below). Bacteremia, inflammation, hemorrhage and necrosis all appeared significantly diminished in incidence and severity (>> 50%) in non-surviving raxibacumab-treated animals compared to placebo. The large reduction of extravascular bacteria in organs in the raxibacumab animals was most striking, with generally a small number of non-surviving animals with low residual levels of bacteria (severity score: 0-0.7), primarily in the draining bronchial and mediastinal lymph nodes. Hepatocellular necrosis of similar incidence but slightly greater severity was observed in raxibacumab-treated animals compared to placebo. Despite the inhalation route of delivery of the spores, the lungs did not appear to be the target organ for anthrax toxicity, with only minor inflammation, no hemorrhage or necrosis, and little fibrin accumulation detected in both placebo and raxibacumab-treated animals. Although important to the initial pathogenesis establishing systemic infection, inhalational anthrax is not considered a primary pulmonary disease¹⁶.

Table 5.1-5: Histopathology Findings of Selected Non-CNS Organs in Non-Surviving Rabbits

Group	Raxibacumab Incidence (Mean Severity)	Placebo Incidence (Mean Severity)
Number In Group	24	24
Mortality	54%	100%
# dead	13	24
Appendix		
Bacteria, Extravascular	0 (0)	6 (1.4)
Hemorrhage	1 (1.0)	6 (1.4)
Necrosis, Lymphoid	1 (3.0)	7 (1.9)
Blood Vessels		
Bacteria, Intravascular	2 (0.3)	20 (2.4)
Raxibacumab (+) Staining, Plasma	13 (3.8)	0 (0)
Kidney		
Bacteria, Extravascular or Glomerular	2 (0.4)	20 (1.8)
Liver		
Bacteria	0 (0)	13 (1.3)
Necrosis, Hepatocellular	8 (1.2)	8 (0.6)
Sinusoidal Leukocytosis	4 (0.4)	13 (1.0)
Lung		
Bacteria, Extravascular or Septal	2 (0.4)	21 (2.3)
Fibrin Accumulation	1 (0.1)	6 (0.5)
Inflammation, Heterophils Predominating	5 (0.7)	14 (0.9)
Lymph Node, Bronchial		
Bacteria, Extravascular or Septal	3 (0.7)	22 (3.0)
Fibrin Accumulation	7 (1.4)	22 (2.3)
Hemorrhage(s)	8 (1.3)	21 (2.3)
Inflammation, Heterophils Predominating	6 (0.8)	21 (2.6)
Necrosis, Lymphoid	8 (1.9)	20 (3.1)
Lymph Node, Mediastinal		
Bacteria, Extravascular or Septal	4 (0.8)	24 (3.6)
Fibrin Accumulation	10 (1.7)	20 (1.9)
Hemorrhage(s)	8 (1.6)	22 (2.1)
Inflammation, Heterophils Predominating	11 (1.5)	22 (2.5)
Necrosis, Lymphoid	10 (2.0)	22 (3.2)
Spleen		
Bacteria	1 (0.3)	22 (3.3)
Fibrin Accumulation	10 (2.0)	11 (1.0)
Inflammation, Heterophils Predominance	6 (0.9)	10 (0.8)

Severity is defined as 1=minimal, 2=mild, 3=moderate, 4=severe

(Table 8-6 on page 44-45 of the HGS study report)

¹⁶ Barnes, J.M. (1947). The development of anthrax following the administration of spores by inhalation. *Br. J. Exp. Pathol.* **28(6)**: 385-394.

Table 5.1-6: Histopathology Findings of Selected Organs in Surviving Rabbits

Group	Raxibacumab Incidence (Mean Severity)
Number In Group	24
Mortality	54%
# survivors	11
Appendix	
Blood Vessels	
Raxibacumab Positive Staining, Plasma	3 (0.4)
Brain	
Acute neurodegeneration, Fluorojade-C	1 (na)
Hemorrhage(s)	1 (0.2)
Hemorrhage(s), meninges	1 (0.2)
Hemorrhage(s), ventricular system	1 (0.1)
Kidney	
Inflammation, heterophils predominating	1 (0.1)
Tubule-interstitial nephritis	1 (0.1)
Liver	
Lung	
Inflammation, chronic/acute chronic	3 (0.4)
Lymph Node, Bronchial	
Hemorrhage(s)	1 (0.1)
Increased macrophage numbers	1 (0.2)
Inflammation, heterophils predominating	5 (0.6)
Hyperplasia, lymphoid tissue	3 (0.4)
Lymph Node, Mediastinal	
Edema	1 (0.1)
Hemorrhage(s)	1 (0.1)
Inflammation, heterophils predominating	5 (0.6)
Spleen	
Hemosiderosis	8 (1.3)
Hyperplasia, lymphoid tissue	6 (0.9)
Increased macrophage numbers	1 (0.1)
Inflammation, Heterophils predominating	8 (1.1)

Severity is defined as 1=minimal, 2=mild, 3=moderate, 4=severe

na: Severity not applicable, severity grade not provided

Table 8-7 on page 46-47 of the HGS study report

Discussion

The current study examined histopathology in raxibacumab-treated and placebo-treated NZW rabbits that either survived or succumbed to a lethal inhalation challenge with *B. anthracis*. Both the estimated survival benefit of raxibacumab monotherapy (46% vs 0% in the placebo group) and the development of CNS lesions with raxibacumab treatment mirrors previous findings in earlier efficacy studies with raxibacumab versus placebo in spore challenged rabbits and non-human primates. A more detailed evaluation of the gross and microscopic evaluation of several different regions of the brain (i.e. cerebellum, cerebral cortex, hippocampus, meninges, midbrain, and thalamus) in surviving and non-surviving rabbits showed that brain lesions (bacteremia, inflammation, hemorrhage, and/or necrosis) occurred predominantly in non-surviving rabbits in the raxibacumab and placebo treatment groups; surviving animals showed no brain lesions or raxibacumab staining of neural tissue and no clinical signs of CNS toxicity.

Natural history studies of inhalational anthrax in rabbits published in the scientific literature note the following “A low incidence of hemorrhage with bacilli occurred in the brain and meninges...of aerosol

exposed rabbits. The lesion in rabbits differed from that seen most often in human or in nonhuman primates in that it was devoid of any accompanying leukocyte infiltrate.”¹⁷

In this study, as in previous raxibacumab monotherapy studies, non-surviving animals, particularly in the raxibacumab treatment group, showed widespread lesions throughout several different regions of the brain with no specific region any more susceptible than another. Both raxibacumab and endogenous rabbit IgG-stained neuropil, neurons, and glial cell types were seen in the brains of non-surviving raxibacumab-treated animals; the similarity of staining patterns suggests non-specific leakage of plasma containing antibodies across a damaged blood brain barrier. The study pathologist noted that parabrachial neurons that stained intensely with both raxibacumab and IgG can be exposed to greater concentrations of circulating immunoglobulins due to their proximity to the ependyma where the BBB is less well-developed. However, the question remains why non-surviving raxibacumab animals showed greater bacteria levels, inflammation, hemorrhage, and necrosis in the brain compared to placebo animals when non-surviving animals from both groups died at a similar time post-treatment. Severe inflammation and hemorrhage in the non-surviving raxibacumab-treated animals that lived longest did not necessarily correlate with highest bacteria levels.

High bacterial load detected in the brains of non-surviving raxibacumab-treated animals suggest a greater proliferation of *B. anthracis* within the brain compared to other organs. It's suspected that raxibacumab is mostly excluded from the CNS in the early stages of the infection until the circulating toxins and bacteria disrupt endothelial cell tight junctions and compromise the integrity of the BBB. In contrast, high plasma concentrations of raxibacumab immediately after treatment significantly diminished bacterial load in all organs other than brain, with marked reduction in incidence and severity of pathologic findings compared to placebo. An in vitro study described in the literature showed that purified anti-PA antibodies can stimulate phagocytic uptake of spores by macrophages; and inhibit spore germination¹⁸. This might explain how raxibacumab could reduce the bacterial load in well-vascularized organs outside of the BBB, and also why bacterial loads remained high in the brain of treated animals. Also, LT-induced immunosuppression may contribute to bacterial invasion and dissemination in all tissues including the brain¹⁹. Reversal of LT immunosuppression with raxibacumab treatment might occur in tissues to which raxibacumab readily distributes; raxibacumab is generally excluded from brain until the BBB is compromised. However, upon compromise of BBB integrity, raxibacumab distribution to the brain and subsequent response of “re-activated” immune cells to proliferating bacteria may help explain the greater CNS lesions in the non-surviving raxibacumab-treated animals compared to placebo.

¹⁷ Zaucha, G.M., Pitt, M.L.M., Estep, J., Ivins, B.E., Friedlander, A.M. (1998). The pathology of experimental anthrax in rabbits exposed by inhalation and subcutaneous anthrax. *Arch.Pathol. Lab. Med.* **122** (11); pg. 982-992.

¹⁸ Welkos, S., Little, S., Friedlander, A., Fritz, D., and Fellows, P. (2001). The role of antibodies to *Bacillus anthracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. *Microbiology*. **147**, 1677-1685.

¹⁹ Sun, C., Fang, H., Xie, T., Auth, R.D., Patel, N., Murray, P.R., Snoy, P.J., Frucht, D.M. (2012). Anthrax lethal toxin disrupts intestinal barrier function and causes systemic infections with enteric bacteria. *Plos One*. **7**(3); e33583-e33583.

5.2 Added Benefit Study for Raxibacumab in Rabbits

Study 1141-CG920871: “Added Benefit of Raxibacumab with Levofloxacin vs. Levofloxacin as Post-Exposure Treatment in the New Zealand White Rabbit Inhalational Anthrax Model”

Objective: To evaluate the added benefit of raxibacumab when administered as a therapeutic agent in combination with levofloxacin at a pre-determined time point against lethality due to inhalation exposure of *B. anthracis* in NZW rabbits.

5.2.1 Study Design

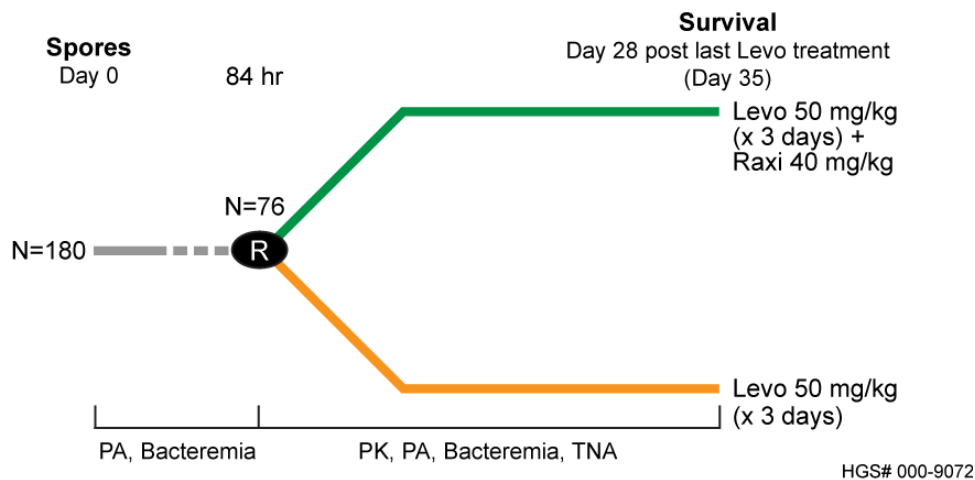
This was a parallel-group, randomized, blinded, GLP efficacy study to evaluate the added benefit of raxibacumab to levofloxacin as therapeutic treatment when administered at 84 hours post challenge in the NZW rabbit inhalational anthrax model. PA kinetics, raxibacumab and levofloxacin PK, and anti-raxibacumab and toxin neutralizing antibodies (TNA) were also examined.

Methods:

The study was conducted at Battelle Biomedical Research Center, West Jefferson, OH. One hundred and eighty (50% male, 50% female), 7-month-old New Zealand White rabbits with surgically implanted vascular access ports (VAP), weighing between 2-5 kg were first randomized into 1 of 6 aerosol challenge days (30 animals/challenge day). Animals were challenged with a targeted 200 LD₅₀ [2.1×10^7 spores] *B. anthracis* (Ames strain) aerosol. Aerosol concentrations of *B. anthracis* were quantified by determination of colony forming units (cfu) in effluent streams. At 84±4 hours post-exposure, surviving animals (N = 76) were further randomized into two groups; levofloxacin + buffer (active control) or levofloxacin + 40 mg/kg raxibacumab (See Figure 5.2-1 below). Levofloxacin (50 mg/kg) was administered by gastric intubation and raxibacumab and buffer were injected intravenously into the VAP. Animals received levofloxacin initially at 84 hours and every 24 ± 1 hours thereafter for another 2 days. The challenge time of 84 ± 4 hrs was chosen to achieve a survival rate similar to that observed (55%) during human anthrax attacks in 2001.

The primary endpoint was survival 28 days after the last dose of levofloxacin. Surviving animals were euthanized on Day 35 post challenge. The study director, technical staff and study pathologists were blinded to treatment until completion of the in-life phase of the study.

Figure 5.2-1: Study Design (Study #: 1141-CG920871)



(Figure 5-1 on page 13 of the HGS study report)

The 40 mg/kg dose of raxibacumab selected is the proposed for licensure (40 mg/kg) and is based on pivotal rabbit and monkey therapeutic efficacy studies, and safety clinical trials in healthy human volunteers. The dose of levofloxacin in this study (50 mg/kg) was chosen to achieve levofloxacin exposures similar or less than observed in humans at the approved doses (between 500 and 750 mg).

Animals were observed every 6 hours from challenge time up to 10 days post-challenge, and then twice daily. Body temperature was monitored twice daily and body weights were collected during quarantine and on study day 0 for calculating dose volumes of test article and control material. Death or euthanasia was recorded at the time observed. Blood samples were collected at several study time points and assayed for bacteremia, plasma PA, plasma raxibacumab and immunoglobulin levels, immunogenicity (anti-raxibacumab antibodies), and toxin neutralizing antibodies (TNA). Complete gross necropsies were conducted on all rabbits. Brain, lungs, spleen, liver, kidney, and mediastinal/bronchial lymph nodes were collected and evaluated in all animals for gross and microscopic findings. The study pathologist was blinded to the treatment administered to each rabbit until after the slides were read. The histologic assessment conducted by the study pathologist was subject to peer review.

5.2.2 Results

Seventy-six animals survived to 84 hours and were randomized to the two treatment arms: 37 to the levofloxacin arm and 39 to the levofloxacin + raxibacumab arm. The two treatment groups were comparable with respect to sex, weight, and age at randomization.

Anthrax Exposure

The mean challenge dose for all 6 challenge days was 188xLD₅₀, and resulted in 58% mortality by the predetermined treatment time (84 hours). Anthrax spore challenge was greater in the levofloxacin + raxibacumab treatment group than the levofloxacin + buffer group (active control). Analyses were conducted to evaluate any effect of the LD₅₀ doses on either survival to day 84, survival rate post treatment, and time to death post treatment and no associations were found.

Table 5.2-1: Average Challenge Doses (LD₅₀ equivalent)

Challenge Day	Average Challenge Dose (SD)
A	176 (± 35)
B	179 (± 45)
C	209 (± 38)
D	184 (± 38)
E	194 (± 48)
F	182 (± 58)
Treatment Group	Average Challenge Dose (SD)
Raxibacumab	197 (± 49)
Raxibacumab Buffer	174 (± 43)
Non-Treated	189 (± 43)

(Adapted from Table 5 on page 22 of the Battelle Study Report)

Mortality and Survival

Of 180 originally challenged animals, 104 succumbed to disease prior to the protocol-defined treatment time (84 ± 4 hrs) and were excluded from the study. Thirty-seven animals were treated with at least one dose of levofloxacin (50 mg/kg) and a single dose of buffer (0.8 mL/kg); thirty-nine animals were treated with at least one dose of levofloxacin (50 mg/kg) and a single dose of raxibacumab (40 mg/kg) (See Table 5.2-2 below).

Table 5.2-2: Animal Disposition

	Levo	Levo/Raxi	Total
Animals challenged			180
Animals Randomized (survived to 84 hours)	37	39	76
Animals Treated	37	39	76
Survived to 28 days after last levofloxacin dose			
Survived	24	32	56
Died	13	7	20
Analysis Population			
Intent-to-Treat (ITT)	37	39	76
Toxic at or before treatment initiation	37	39	76
Bacteremic at or before treatment initiation	37	38*	75

*L34828 not bacteremic during the entire study, survived.

FDA table

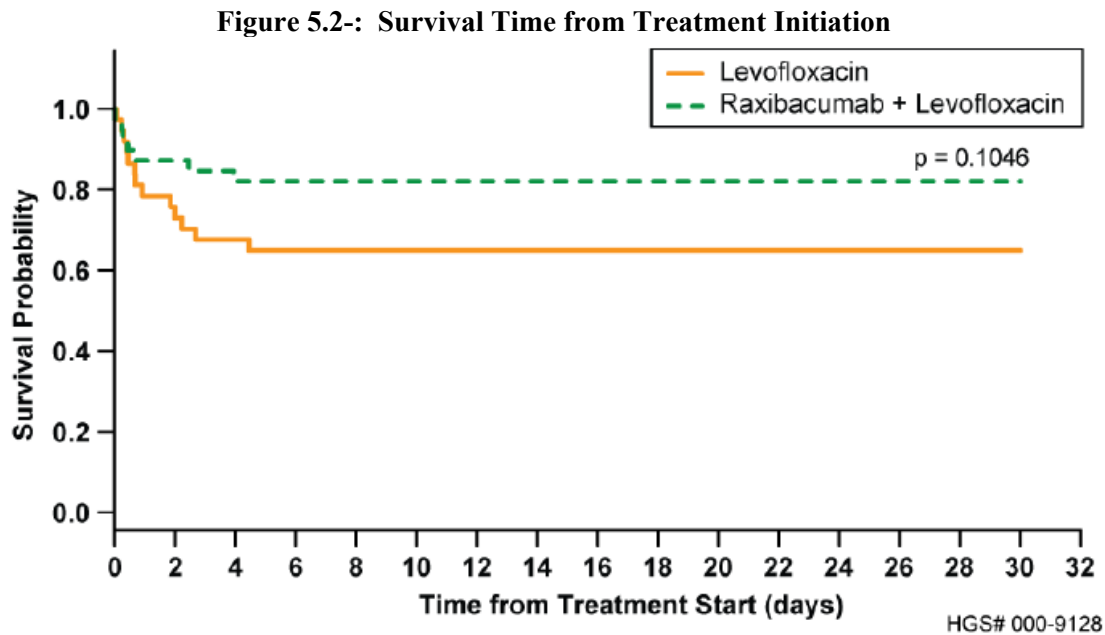
Of the 37 animals treated with levofloxacin and buffer, 24 animals (64.9%) survived to Study Day 35 after the last dose of levofloxacin. Of the 39 animals treated with levofloxacin and raxibacumab, 32 animals (82.1%) survived through Study Day 28. Although not statistically significant (p=0.0874), a trend towards greater survival (17% treatment difference) was observed when both levofloxacin and raxibacumab were administered (See table 5.2-3 below).

Table 5.2-3: Day 28 Survival Rate (FDA analysis)

Study	Levo	Levo/Raxi	Difference (Levo/Raxi – Levo) (95% CI)	<i>P</i> -value ¹
ITT animals	24/37 (64.9%)	32/39 (82.1%)	17.2 (-2.4, 36.7)	0.0874
Bacteremic at or before treatment	24/37 (64.9%)	31/38 (81.6%)	16.7 (-3.0, 36.4)	0.0998

¹ 2-sided likelihood ratio chi-square test

The following figures shows the survival curves for randomized animals (see Figure 5.2-2 below).



(Figure 1 from Battelle Study Report, pg 23).

In examining the subpopulation of non-surviving animals that died prior to Day 28 post-treatment, the mean time from spore challenge to death was found to be similar, 4.9 days in the levofloxacin alone compared to 4.7 days in the levofloxacin + raxibacumab treatment group. Animals that survived until Day 9 post-challenge in either treatment group remained alive until the scheduled terminal euthanasia on Day 35. The TTD listed is an estimate of the time to death post-treatment since a majority of dead animals in both groups were found dead in their cages.

Blood Culture Levels of *B. anthracis*

Qualitative blood cultures were positive in 179/180 animals challenged; 75/76 (98%) of animals that were treated at 84 ± 4 hrs were positive for bacteremia prior to treatment (37 randomized to levofloxacin and 38 randomized to levofloxacin + raxibacumab. For the levofloxacin + raxibacumab treatment group, within 2 hours after the first dose of levofloxacin, 16/39 (41%) animals were already negative for bacteremia. For the levofloxacin alone group, within 2 hours after the first dose of levofloxacin 10/37 (27%) had cleared bacteremia. At 24 hours post-treatment, 32 (82%) animals treated with levofloxacin + raxibacumab and 29 (78%) animals treated with levofloxacin + buffer were negative for bacteria in blood cultures. Two animals with positive blood cultures 24 hours after the first dose of levofloxacin +

raxibacumab (L35259 and L35261) were negative for blood cultures 2 hours after receiving the second dose of levofloxacin.

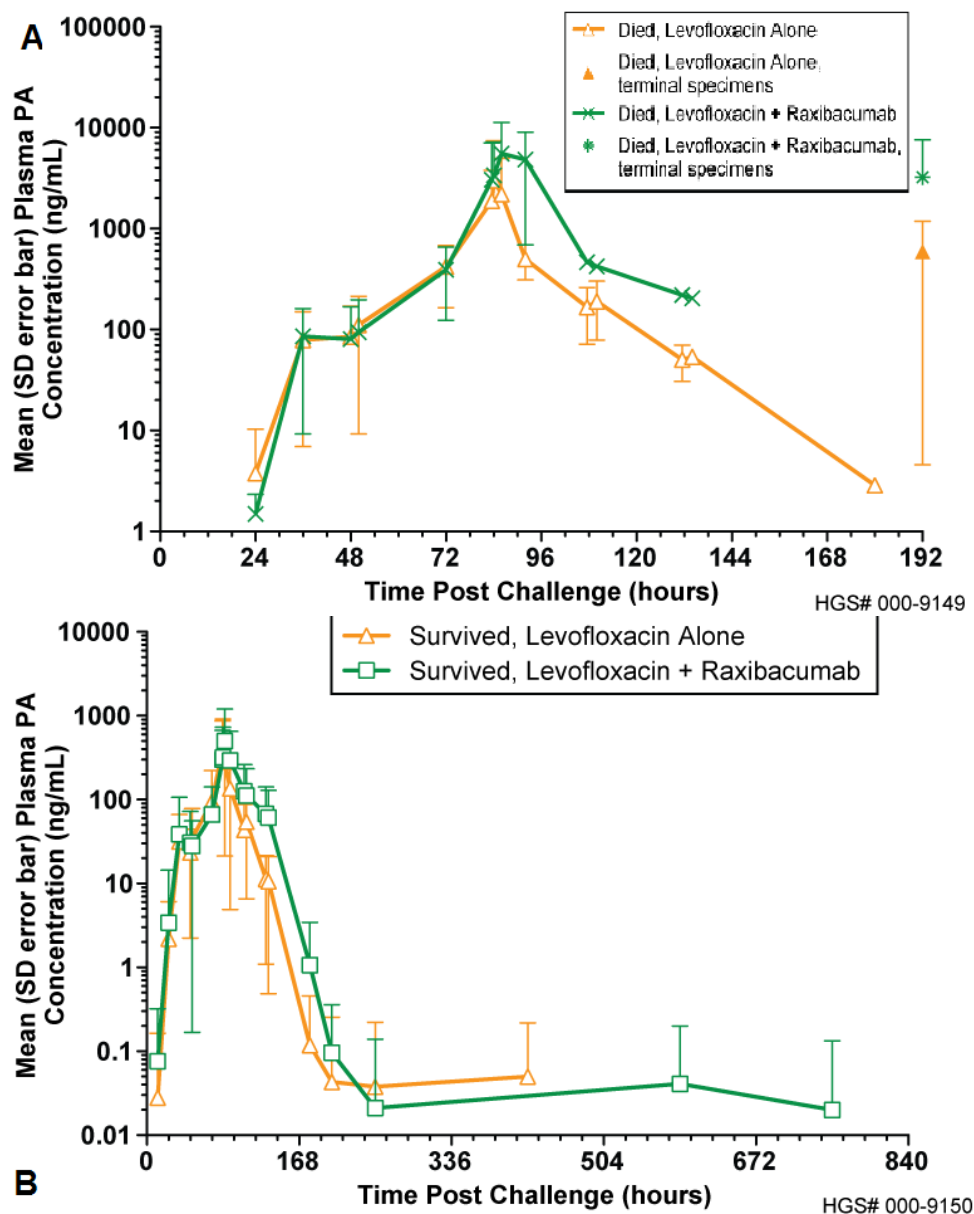
Of the 13 animals treated with raxibacumab buffer that died, 2 animals were bacteremic at death. Of the 7 animals treated with raxibacumab that died, 3 animals were bacteremic at death.

Quantitative Plasma PA Results

No animals were PA positive pre-challenge. The applicant's mean plasma PA concentration-time profiles for the dead (Figure 5.2-5A) and surviving (Figure 5.2-5B) animals treated with levofloxacin vs. levofloxacin+40 mg/kg raxibacumab are shown below. Comparison of the PA kinetics for treated rabbits that died and survived showed a general overlap in PA kinetic estimates, with the exception of both the height and duration of the first and second plateau in PA levels. Animals that survived to 84 hours post-challenge had 10-fold less plasma PA than non-surviving animals at this time point. Declining plasma PA levels after the timing of the second plateau were mostly associated with negative bacteremia results. For all surviving animals in both treatment groups, plasma PA concentrations declined below the LLOQ by the end of the study. The decrease in plasma PA concentrations may be the result of raxibacumab-PA complex formation and decreased detection in the assay, or from decreased bacteremia via increased bacterial clearance or inhibition of spore germination²⁰.

²⁰ Welkos, S., Little, S., Friedlander, A., Fritz, D., and Fellows, P. (2001). The role of antibodies to *Bacillus anthracis* and anthrax toxin components in inhibiting the early stages of infection by anthrax spores. *Microbiology*. **147**, 1677-1685.

Figure 5.2-3: Plasma PA Concentration-Time Profile in Treated Rabbits that Died (A) and Survived (B)



(Adapted from figures 5-7 and 5-8 on page 402-403 of the Battelle study report)

Raxibacumab Pharmacokinetics

Raxibacumab was not detected in the pre-dose specimens. The mean observed raxibacumab concentration-time profiles were similar to those observed in the original monotherapy and combination studies with levofloxacin in rabbits.

Levofloxacin Pharmacokinetics

No measurable plasma levofloxacin concentrations were detected in any pre-dose specimens. Co-administration of raxibacumab had no effect on plasma concentrations of levofloxacin at any time point. PK analysis of levofloxacin in animals dosed with levofloxacin oral solution by intragastric gavage once daily for 3 days in the presence or absence of a single IV dose of raxibacumab (40 mg/kg) showed levofloxacin exposure similar or lower than exposures achieved in humans at the approved 500 or 750 mg levofloxacin dose as described in the Levaquin® package insert (Table 5.2-4).

Table 5.2-4: Levofloxacin Exposures for Rabbits in the Current Study Compared with Human Exposures Administered 500 or 750 mg Levofloxacin Doses (in the Labeling)

Levofloxacin Dosing	C _{max,n} (µg/mL)	C _{min,n} (µg/mL)
Rabbit, based on PK results from current study ¹		
1 st Dose	6.2	0.4
2 nd Dose	7.5	0.5
3 rd Dose	7.0	0.8
Human, based on information provided in Levaquin® product labeling		
500 mg	5.7	0.5
750 mg	8.6	1.1

Abbreviations: C_{max,n}, maximum plasma levofloxacin concentration after the nth dose, defined as the concentration measured 2 hours after the dose; C_{min,n}, minimum plasma levofloxacin concentration after the nth dose, defined as the concentration measured just prior to the subsequent dose, or at 24 hours after the 3rd dose.

¹ Mean values for Group 2 (levofloxacin alone).

Sources: [Appendix 10](#) and Levaquin® package insert, 2011.

(Table 5-2 on page 388 of the Battelle study report)

Immunogenicity (Anti-Raxibacumab Antibodies) and TNA Titer Results

Plasma samples were assessed for anti-raxibacumab antibodies using a 2-part ECL-based assay including a preliminary screening assay and an inhibition of binding assay to confirm antibody positivity. The overall incidence of anti-raxibacumab antibody in the raxibacumab-treated group at Day 28 post-treatment was 23/32 (72%), excluding the 7 non-survivors for whom immunogenicity outcome is unknown. Three (8%) of the raxibacumab-treated animals were positive for anti-raxibacumab antibody prior to spore challenge and on Day 28 post-treatment. The remaining 20 surviving animals that were positive for raxibacumab antibody in the raxibacumab group on Day 28 had negative results prior to spore challenge.

TNA titers were similar at 28 days post treatment in levofloxacin alone and levofloxacin + raxibacumab treated animals. TNA was not detected prior to spore challenge. Raxibacumab did not interfere with development of endogenous toxin neutralizing antibodies (TNA); high TNA titers ($\mu \approx 5000$) detected only in surviving rabbits 28-days post-treatment were generated in the presence of high plasma raxibacumab levels after treatment.

5.2.3 Necropsy and Histopathology

Complete necropsies were performed on animals found dead or euthanized, including animals surviving to terminal euthanasia on Day 28 post-treatment. Gross lesions in rabbits found dead or euthanized moribund include discoloration or foci in the appendix and the brain (hemorrhage and inflammation), enlargement of bronchial and mediastinal lymph nodes (edema, fibrin exudation, hemorrhage), and fluid

(effusion) in the body cavities and thymus. These findings were considered by the reviewing pathologist to be typical of anthrax infection in rabbits. Microscopic findings consistent with anthrax were present in all rabbits that died or became moribund during the study including acute suppurative inflammation, necrosis, hemorrhage, edema, and vasculitis in the appendix, brain, kidney, liver, lung, bronchial and mediastinal lymph nodes, spleen, and thymus (Table 5.2-5). Anthrax related findings in the brain (meningeal and parenchymal hemorrhage and meningeal vascular necrosis) were present in only two non-survivors treated with levofloxacin alone. No abnormalities on brain histopathology were noted in any animals in the levofloxacin + raxibacumab group. Large rod-shaped bacteria characteristic of *B. anthracis* were present in the brain, lung, lymph nodes, and spleen of three levofloxacin animals and one levofloxacin + raxibacumab animal that died during the study; the remaining treated animals that died before Day 35 in both groups lacked visible bacteria in any organs examined. All dead animals, however, were bacteremic at the time of death with associated inflammation and/or hemorrhage typical of anthrax infection in one or more of the organs examined. In contrast, there were no anthrax-related microscopic findings in any animals that survived until Day 35.

Table 5.2-7: Incidence and mean severity of selected microscopic observations in non-surviving animals

Tissue/Observation		Levofloxacin (50 mg/kg x 3) + Placebo Incidence (mean severity) N = 13	Levofloxacin (50 mg/kg x 3) + Raxibacumab (40 mg/kg) Incidence (mean severity) N = 7
Brain	Number Examined	13	6
	Bacteria, meninges	1 (0.2)	0 (0)
	Hemorrhage(s)	2 (0.3)	0 (0)
	Hemorrhage(s), Cerebellum	1 (0.1)	0 (0)
	Hemorrhage(s), Cerebral Cortex, Fronto-Parietal	2 (0.2)	0 (0)
	Hemorrhage(s), Cerebral Cortex, Temporo-Parietal	1 (0.1)	0 (0)
	Hemorrhage(s), Meninges	1 (0.2)	0 (0)
	Hemorrhage(s), Thalamus	1 (0.1)	0 (0)
	Necrosis, Meningeal vascular	1 (0.2)	0 (0)
	Necrosis, Parenchyma	1 (0.1)	0 (0)
Kidney	Number Examined	13	7
	Tubular Atrophy	1 (0.2)	0 (0)
Liver	Number Examined	13	7
	Necrosis, Hepatocellular	6 (0.7)	2 (0.6)
	Sinusoidal Leukocytosis	2 (0.2)	1 (0.1)
Lung	Number Examined	13	7
	Bacteria	2 (0.4)	-
	Hemorrhage	5 (0.7)	2 (0.3)
	Necrosis, BALT	4 (0.9)	1 (0.6)
	Fibrin exudation, alveolar	4 (0.7)	2 (0.3)
	Heterophilic inflammation	4 (0.7)	1 (0.3)
Lymph Node, Bronchial	Number Examined	13	7
	Bacteria	2 (0.3)	0 (0)
	Fibrin exudation	9 (1.6)	5 (2.3)
	Hemorrhage(s)	8 (0.9)	5 (0.9)
	Heterophilic inflammation	2 (0.3)	4 (0.9)
	Necrosis, lymphoid	11 (2.6)	6 (2.6)
Lymph Node, Mediastinal	Number Examined	13	7
	Bacteria	2 (0.5)	1 (0.3)
	Fibrin exudation	8 (1.6)	5 (1.6)
	Hemorrhage(s)	8 (1.2)	5 (1.1)
	Heterophilic inflammation	1 (0.2)	3 (0.6)
	Necrosis, lymphoid	11 (2.3)	5 (2.1)
Spleen	Number Examined	13	6
	Bacteria	0 (0.0)	1 (0.2)
	Fibrin exudation	4 (0.5)	3 (0.8)
	Heterophilic inflammation	2 (0.2)	1 (0.3)
	Necrosis, lymphoid	3 (0.6)	1 (0.3)

- no value given

Source: Table 7 and Table 8, Appendix Q of the Battelle study report

Discussion

The primary objective of this study was to assess the added benefit of raxibacumab to levofloxacin treatment of inhalation *B. anthracis* exposure to prevent lethality in rabbits when administered in combination at 84 hours post-spore challenge. Previous nonclinical combination therapy studies with raxibacumab and levofloxacin in rabbits administered to inhalational anthrax challenged animals immediately after detection of systemic anthrax disease showed no difference in survival rates ($\approx 95\%$) between levofloxacin and levofloxacin/raxibacumab combination groups. To address the added benefit concern included in FDA's Complete Response Letter (2009) sent to the sponsor, HGS conducted a nonclinical combination treatment study in the inhalational anthrax rabbit model in which treatment with levofloxacin and levofloxacin + raxibacumab was delayed to 84 hours post-challenge. (The protocol-defined challenge time of 84 ± 4 hrs was chosen to achieve a survival rate ($\approx 55\%$) similar to that observed during human anthrax attacks in 2001).

In this study, 76/180 (42%) spore-challenged animals survived to 84 hours post-challenge, the pre-set intervention time. Nearly all (75/76) of the animals that survived to 84 hours post-challenge were positive for bacteremia prior to treatment. After randomization of survivors, combination treatment with levofloxacin + raxibacumab showed a positive trend toward greater survival of $\approx 17\%$ ($p=0.0874$) compared to levofloxacin alone. This study was not designed to be adequately powered to detect a statistically significant difference between the study arms. Raxibacumab had no clear impact on time from spore challenge to death for non-surviving animals in both treatment groups (identical mean time to death of 4.7 to 4.9 days). No animals died beyond 3 days after the last dose of levofloxacin. There is no significant difference in survival time from the initiation of spore challenge between the raxibacumab/levofloxacin combination group and the levofloxacin group.

Clearance of bacteremia at 24 hours after start of treatment was similar in both surviving and non-surviving animals. By 24 hours post-treatment, $> 75\%$ of animals in both treatment groups had negative blood cultures. Raxibacumab + levofloxacin showed a trend toward a slightly faster bacterial clearance at 2 hours after start of treatment than levofloxacin alone. Co-administration of raxibacumab and levofloxacin had no effect on plasma concentrations and PK profiles of either product at any time point. Raxibacumab and levofloxacin PK profiles for each drug appeared similar to previous studies despite the 84 hour delay in treatment. Levofloxacin at 50mg/kg administered to rabbits resulted in plasma exposures similar or lower than exposures achieved in humans at the approved 500 or 750 mg levofloxacin dose.

Anti-raxibacumab antibodies were detected in a majority (23/32) of animals in the raxibacumab + levofloxacin treatment group. Pre-existing antibodies to raxibacumab were detected in 3 of these animals. Increased clearance of raxibacumab detected in survivors > 7 days post-challenge likely resulted from binding to anti-drug antibodies. Despite this, the greater survival rate in the raxibacumab treatment group indicates no significant impact of drug-antibody binding on efficacy of raxibacumab in the treated survivors. Similarly, survivors in both treatment groups developed high plasma titers of toxin neutralizing antibodies (TNA) occurred. The innate and/or acquired immune response in rabbits to PA and other anthrax toxins appear unaffected by either treatment despite rapid clearance of bacteremia.

No gross or microscopic findings attributable to spore challenge or treatment were observed in surviving animals in both treatment groups. Non-surviving animals were determined to have died with lesions characteristic of inhalational anthrax exposure, including hemorrhage, inflammation, necrosis, edema, and effusion of critical organs. Interestingly, there were no findings on brain histopathology in any levofloxacin + raxibacumab treated animals. Brain findings such as meningeal and parenchymal hemorrhage and meningeal vascular necrosis were noted only in 2 non-survivors treated with levofloxacin

alone. Clearance of bacteria was observed in the brain, lung, lymph nodes, and spleen of a few animals in both groups; a majority of animals showed no visible bacteria in any organs examined. Levofloxacin mediated elimination of visceral bacteria appears to reduce the severity of anthrax-related lesions, particularly increased CNS lesions (hemorrhage, inflammation, bacteria, and necrosis) observed in the brains of raxibacumab-treated animals in previous monotherapy trials.

Although a positive, statistically significant added benefit result was not achieved with this under-powered study design, there appears to be a trend towards greater survival in rabbits when raxibacumab is co-administered with levofloxacin 84 hours after inhalational anthrax exposure. Also, raxibacumab has been shown not to interfere with levofloxacin efficacy or with the innate or acquired immune response to bacteria and/or bacterial toxins.

6. Clinical Pharmacology

6.1 *Interspecies Comparison of Raxibacumab Pharmacokinetics*

To allow selection of an effective dose in humans for therapeutic treatment of anthrax, the applicant has submitted clinical pharmacology data for raxibacumab in humans and in the two pivotal animal species, NZW rabbits and cynomolgus macaques. Pharmacokinetic information for raxibacumab following intravenous administration was evaluated in humans, monkeys and rabbits in the following studies and was used for purposes of interspecies comparison:

- Study HGS1021-C1064: an open-label study designed to determine the effect of co-administration of raxibacumab on PO administered ciprofloxacin PK and characterize the effect of co-administration of PO and IV administered ciprofloxacin on raxibacumab PK in healthy human subjects.
- Study 724-G005829: an animal efficacy study designed to evaluate raxibacumab efficacy as therapeutic treatment against inhalational anthrax in the monkey model; this study included an evaluation of the PK of raxibacumab and the kinetics of *Bacillus anthracis* PA following a single IV raxibacumab dose in monkeys with inhalational anthrax (Pivotal Monkey Efficacy Study).
- Study 682-G005758: an animal efficacy study designed to evaluate raxibacumab efficacy as therapeutic treatment against inhalational anthrax in the rabbit model; this study included an evaluation of the PK of raxibacumab and the kinetics of *Bacillus anthracis* PA following a single IV raxibacumab dose in rabbits with inhalational anthrax (Pivotal Rabbit Efficacy Study).

Serum concentration-time profiles for raxibacumab following single intravenous administration of 40 mg/kg in healthy, male and female subjects, monkeys with inhalational anthrax, and rabbits with inhalational anthrax are presented in Figure 6.1-1. Pharmacokinetic parameters for raxibacumab following single intravenous administration of 40 mg/kg in healthy, male and female subjects, monkeys with inhalational anthrax, and rabbits with inhalational anthrax are summarized in Table 6.1-1.

Figure 6.1-1: Mean (SD) Serum Concentration-Time Profiles for Raxibacumab Following Single Intravenous Administration of 40 mg/kg in Healthy, Male and Female Subjects, Monkeys with Inhalational Anthrax, and Rabbits with Inhalational Anthrax (FDA analysis)

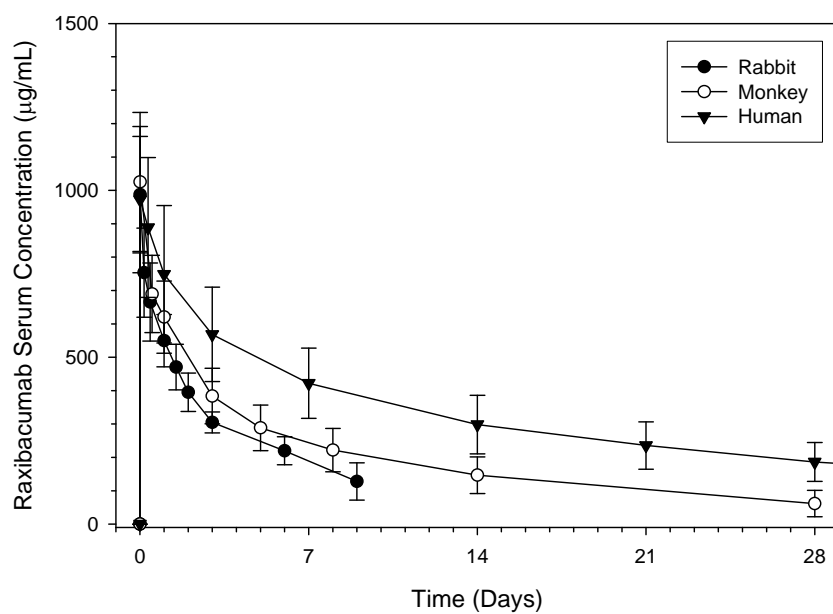


Table 6.1-1: Pharmacokinetic Parameters for Raxibacumab Following Single Intravenous Administration in Healthy, Male and Female Subjects, Monkeys with Inhalational Anthrax, and Rabbits with Inhalational Anthrax (FDA analysis)

Species	Dose Group	N	Cmax	AUCinf	Half life	CL	Vss
			(µg/ml)	(µg-day/ml)	(days)	(ml/day/kg)	(ml/kg)
Rabbit	20 mg/kg	17	459.9 ± 57.8 (342.5 - 544.8)	1719.1 ± 223.5 (1380.4 - 2196.7)	3.97 ± 0.73 (2.63 - 5.44)	11.6 ± 1.71 (9.10 - 15.5)	63.11 ± 7.91 (52.9 - 82.0)
Rabbit	40 mg/kg	19	918.8 ± 124.0 (623.8 - 1166.0)	3424.0 ± 464.1 (2541.4 - 3348.7)	4.10 ± 0.85 (2.99 - 6.10)	11.7 ± 1.81 (8.83 - 15.23)	63.8 ± 8.48 (51.9 - 87.9)
Monkey	20 mg/kg	14	490.5 ± 87.7 (275.6 - 613.2)	3575.9 ± 827.9 (2499.0 - 5240.4)	11.1 ± 1.94 (7.95 - 14.61)	5.85 ± 1.29 (3.80 - 8.00)	84.2 ± 10.3 (73.8 - 110.3)
Monkey	40 mg/kg	14	989.8 ± 170.1 (788.8 - 1422.9)	6490.6 ± 2095.5 (3899.4 - 11934.5)	10.1 ± 2.35 (5.98 - 14.3)	6.61 ± 1.90 (3.30 - 10.2)	82.8 ± 9.10 (64.5 - 93.3)
Human Group 1	40 mg/kg + cipro PO	27 ^a	1143.3 ± 169.5 (833.6 - 1517.8)	14871.9 ± 3821.1 (5494.5 - 21116.6)	19.8 ± 8.18 (4.27 - 45.8)	2.99 ± 1.38 (1.85 - 7.30)	64.9 ± 11.9 (44.1 - 99.8)
Human Group 2	40 mg/kg	27 ^b	1020.3 ± 140.6 (766.2 - 1293.7)	15845.8 ± 4333.5 (7614.9 - 25464.6)	20.6 ± 6.54 (5.93 - 32.3)	2.73 ± 0.84 (1.57 - 5.24)	69.7 ± 13.7 (45.6 - 106.7)
Human Group 3	40 mg/kg + cipro IV/PO	28	1047.8 ± 180.3 (772.5 - 1458.4)	16349.3 ± 4255.7 (7117.5 - 27465.2)	21.5 ± 8.92 (6.53 - 42.9)	2.63 ± 0.82 (1.46 - 5.62)	67.2 ± 12.6 (47.6 - 93.2)

Data presented represents mean ± one standard deviation and (minimum – maximum)

^a Subjects US003-000006 and US003-000028 excluded for receiving only partial doses of raxibacumab. Subject US003-00029 excluded for PK profile uncharacteristic of IV administration.

^b Subject US003-000002 excluded for receiving only a partial dose of raxibacumab.

Serum concentrations of raxibacumab following a single IV dose of 40 mg/kg alone in humans fell within or exceeded the range of concentrations observed in rabbits and monkeys receiving single 40 mg/kg IV doses, except for two subjects at the 24-hour sampling time point. Mean raxibacumab C_{max} in humans following a single 40 mg/kg IV dose was similar to or greater than mean C_{max} values in monkeys and rabbits. Individual raxibacumab C_{max} values in humans following a single IV dose of 40 mg/kg alone fell within the range of C_{max} values observed in rabbits. All but one C_{max} in humans fell within the range of values observed in the monkeys. Mean raxibacumab AUC_{inf} in humans following a single 40 mg/kg IV dose was 2.4- and 4.6-fold that of the mean C_{max} values in monkeys and rabbits, respectively. Individual raxibacumab AUC_{inf} values in humans following a single IV dose of 40 mg/kg alone fell within or exceeded the range of AUC_{inf} values observed in rabbits and monkeys. Clearance of raxibacumab was significantly slower in humans as compared to rabbits, by a factor of approximately 5, and in monkeys, by a factor of approximately 3. Thus, the half-life of raxibacumab was substantially longer in humans compared to mean half-lives observed in monkeys and rabbits (20.6 ± 6.5 days versus 10.1 ± 2.4 days and 4.1 ± 0.85 days, respectively). Variability in C_{max} was similar across species; %CV values ranged between approximately 13 to 17%. Variability in AUC_{inf} was wider, ranging between 13 and 32% across species.

In summary, humans achieve similar or greater exposure to raxibacumab following a single 40 mg/kg IV dose compared to rabbits and monkeys receiving the same dose. Humans achieve much greater exposure to raxibacumab following a single 40 mg/kg IV dose compared to rabbits and monkeys receiving a 20 mg/kg dose.

6.2 Drug Interactions

In the course of treatment of a *B. anthracis* infection and in response to an anthrax-related emergency, raxibacumab will likely be administered concurrently with an antimicrobial agent, such as a fluoroquinolone active against *B. anthracis*. To assess pharmacokinetics and safety of coadministration of a fluoroquinolone antimicrobial and raxibacumab, a Phase 1 drug interaction study with raxibacumab and ciprofloxacin in healthy subjects (HGS1021-C1064) was conducted.

HGS1021-C1064 was an open-label study to evaluate the effect of raxibacumab on ciprofloxacin PK as well as the safety and PK of raxibacumab in combination with ciprofloxacin in healthy adult male and female subjects. Three treatment groups were evaluated. Group 1 received PO ciprofloxacin (500 mg Q12h, Days 0 to 7), with a single raxibacumab (40 mg/kg) dose IV on Day 5. Group 2 received a single raxibacumab (40 mg/kg) dose IV on Day 0. Group 3 received a single IV ciprofloxacin (400 mg) dose on Day 0 immediately followed by a single IV raxibacumab (40 mg/kg) dose, a second IV ciprofloxacin (400 mg) dose 12 hours later, and then PO ciprofloxacin (500 mg Q12h, Days 1 to 7) for a total of 13 doses.

Pharmacokinetic parameters for raxibacumab following single intravenous administration of 40 mg/kg with and without coadministration of ciprofloxacin PO (Group 1) or IV/PO (Group 3) in healthy, male and female subjects are summarized in Table 6.2-1. Serum raxibacumab concentration-time profiles were very similar among treatment groups, with overlapping SD error bars. Statistically significant differences in PK parameters were not encountered for Group 3 (raxibacumab + IV ciprofloxacin) versus the raxibacumab alone group. Overall, exposure to ciprofloxacin appears to have no consistent or meaningful impact on raxibacumab PK.

Table 6.2-1: Summary of Raxibacumab Concentrations Following a Single 40 mg/kg Raxibacumab IV Infusion Administered with or without PO or IV Ciprofloxacin (FDA analysis)

Parameter	Group 1 ^a (n = 27)	Group 2 ^b (n = 27)	Group 3 (n = 28)
C_{max} (µg/ml)	1143.3 ± 169.5 (833.6 – 1517.8)	1020.3 ± 140.6 (766.2 – 1293.7)	1047.8 ± 180.3 (772.5 – 1458.4)
AUC_{inf} (µg·day/ml)	14871.9 ± 3821.1 (5494.5 – 21116.6)	15845.8 ± 4333.5 (7614.9 – 25464.6)	16349.3 ± 4255.7 (7117.5 – 27465.2)
Half life (days)	19.8 ± 8.18 (4.27 – 45.8)	20.6 ± 6.54 (5.93 – 32.3)	21.5 ± 8.92 (6.53 – 42.9)
CL (ml/day/kg)	2.99 ± 1.38 (1.85 – 7.30)	2.73 ± 0.84 (1.57 – 5.24)	2.63 ± 0.82 (1.46 – 5.62)
V_{ss} (ml/kg)	64.9 ± 11.9 (44.1 – 99.8)	69.7 ± 13.7 (45.6 – 106.7)	67.2 ± 12.6 (47.6 – 93.2)

Group 1, raxibacumab 40 mg/kg + cipro PO; Group 2, raxibacumab 40 mg/kg only; Group 3, raxibacumab 40 mg/kg + cipro IV/PO
Data presented represents mean ± one standard deviation and (minimum – maximum)

^a Subjects US003-000006 and US003-000028 excluded for receiving only partial doses of raxibacumab. Subject US003-00029 excluded for PK profile uncharacteristic of IV administration.

^b Subject US003-000002 excluded for receiving only a partial dose of raxibacumab.

Pharmacokinetic parameters for ciprofloxacin following oral administration and IV followed by oral administration with and without coadministration of a raxibacumab 40 mg/kg single dose in healthy, male and female subjects are summarized in Table 6.2-2.

Table 6.2-2: Summary of Ciprofloxacin Pharmacokinetic Parameters Following Oral Administration (Group 1) or Intravenous Followed by Oral Administration (Group 3) with and without a Single Intravenous Dose of Raxibacumab 40 mg/kg in Healthy, Male and Female Subjects (FDA analysis)

Parameter	Group 1		Group 3	
	PO without Raxibacumab (n = 30)	PO with Raxibacumab (n = 30)	IV with Raxibacumab (n = 28)	PO with Raxibacumab (n = 28)
C_{max} (ng/ml)	NA	NA	1854 (402)	NA
C_{ss,max} (ng/ml)	1436 (519)	1419 (599)	NA	1195 (566)
AUC_{inf} (ng·hr/ml)	NA	NA	8770 (1877)	NA
AUC_{tau} (ng·hr/ml)	7694 (2680)	8151 ^a (2673)	NA	6615 (3224)
Half life (hr)	4.74 (2.09)	5.25 ^a (2.47)	4.53 (0.89)	4.62 ^b (1.07)
CL or CL/F (L/hr)	72.4 (24.0)	66.8 ^a (21.9)	47.6 (9.8)	91.1 (36.9)
V_{ss} (L)	NA	NA	285.9 (725.9)	NA
V_z or V_z/F (L)	510.7 (340.3)	486.0 ^a (212.8)	312.0 (933.7)	630.7 ^b (308.6)

Data presented as mean (SD).

Group 1, raxibacumab 40 mg/kg + cipro PO, Group 3, raxibacumab 40 mg/kg + cipro IV/PO

^a n = 29

^b n = 27

Source: HGS1021-C1064 Pharmacokinetic Report, Section 3.5.1.4

Upon statistical comparison for $C_{ss,max}$ and AUC_{τ} , the 90% CI fell within the 80% to 125% range, demonstrating that for those primary parameters Dose 11 (with raxibacumab) was equivalent to Dose 9 (prior to raxibacumab administration). In summary, there is no interaction between raxibacumab and ciprofloxacin; ciprofloxacin exposure is equivalent for ciprofloxacin administered alone and when administered with raxibacumab.

6.3 Exposure-Response for Efficacy

Exposure-response (E-R) analyses for raxibacumab efficacy were performed by the applicant and the FDA. The following is a discussion of the analyses performed by the FDA. Survival rates by raxibacumab dose in the pivotal animal efficacy studies are summarized in Figure 6.3-1. Survival rates in both studies exhibited a dose-response for 20 mg/kg and 40 mg/kg doses of raxibacumab IV. Mirroring the dose-response seen with 20 and 40 mg/kg doses of raxibacumab, a concentration-response is seen between the probability of survival and quantiles of raxibacumab C_{max} , as presented in Figure 6.3-2. The data suggest a relationship between raxibacumab dose, concentration, and survival. Although it is proposed that a sufficiently high C_{max} optimizes the likelihood of efficacy, a threshold of C_{max} could not be identified with the available data.

Figure 6.3-1: Percent Survival in Rabbits and Monkeys Treated with Raxibacumab for Anthrax (FDA analysis)

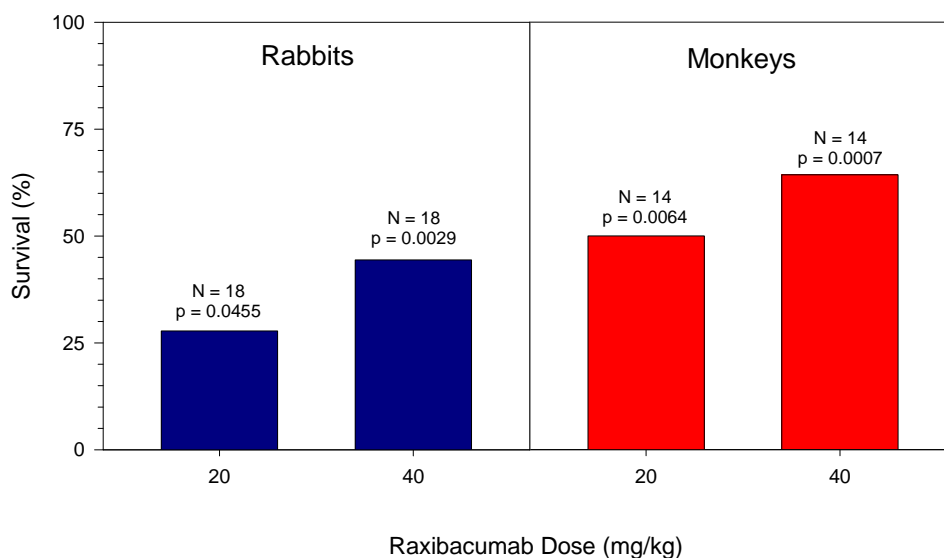
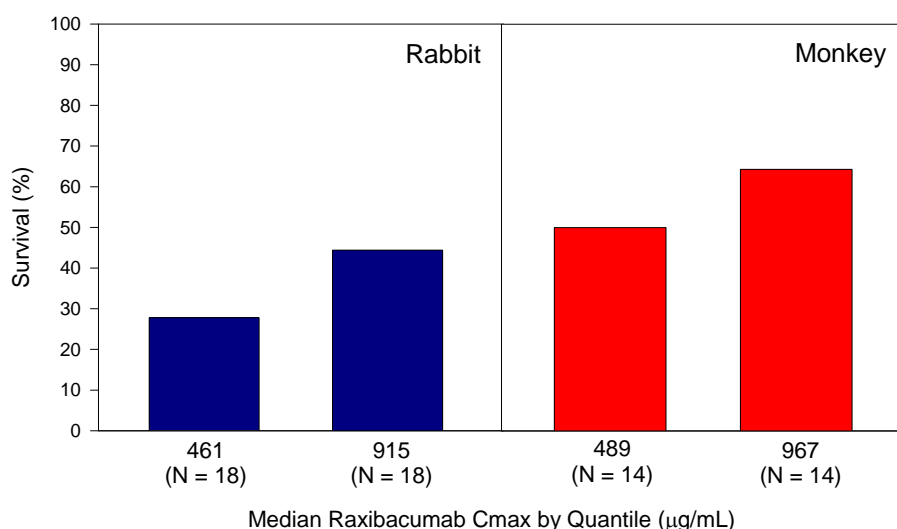


Figure 6.3-2: Comparison of Probabilities of Survival by Raxibacumab Cmax Quantile (FDA analysis)



The definition of an exposure-response relationship between raxibacumab and protective antigen (PA) concentrations is confounded by the following factors: 1) for both analytes, only total serum/plasma concentrations were measured, 2) the measurement of PA concentrations is affected by the presence of raxibacumab in serum and plasma, and 3) significant inherent intra- and inter-individual pharmacodynamic variability of PA data exists. A reasonable assessment of the inter-relationship between raxibacumab concentrations, PA concentrations and survival for purposes of predicting the acceptability of a proposed dose is a comparison of raxibacumab serum concentrations over time to the concentrations required for 99.0 and 99.9% binding of PA (based on the known mechanism of action and binding kinetics of raxibacumab). Based on in vitro binding kinetics studies, serum raxibacumab concentrations of approximately 40 and 202 µg/mL are required for 99.0 and 99.9% binding of PA, respectively. As presented in Figure 6.3-3, in human subjects that received 40 mg/kg raxibacumab IV, raxibacumab concentrations remained > 202 mcg/mL for 7 days and > 40 mcg/mL for 42 days for all human subjects. Thus, in humans, a 40 mg/kg dose of raxibacumab would be expected to maintain levels required for virtually complete binding of PA for 7 days.

Despite the theoretical importance of targeting virtually complete binding of PA, the duration of time raxibacumab concentrations remain above the threshold of 202 mcg/mL does not appear to impact efficacy, as displayed in Figures 6.3-4 and 6.3-5. In both rabbits and monkeys, the amount of time serum concentrations of raxibacumab remained above 202 mcg/mL generally did not differ between survivors and non-survivors.

Figure 6.3-3: Individual Serum Concentrations of Raxibacumab in Human Subjects Following Administration of 40 mg/kg IV Compared to the Concentrations Required for 99 and 99.9% Binding of PA (FDA analysis)

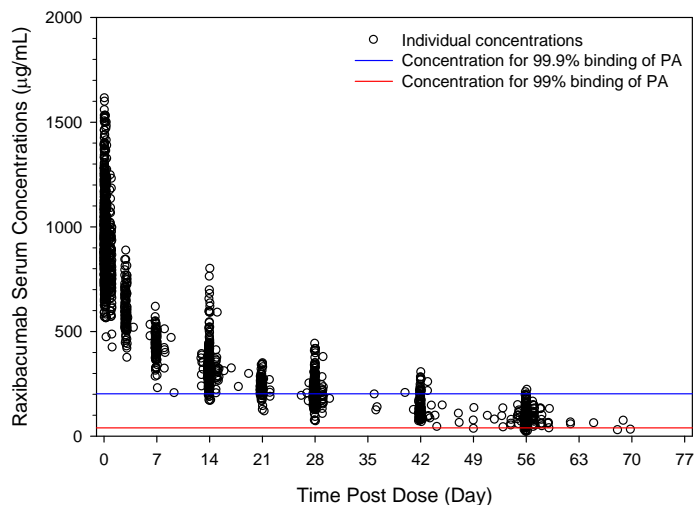


Figure 6.3-4: Spaghetti Plot of Serum Concentration-Time Profiles for Raxibacumab Following Single Intravenous Administration of 20 mg/kg and 40 mg/kg Raxibacumab in Spore-Challenged Rabbits (FDA analysis)

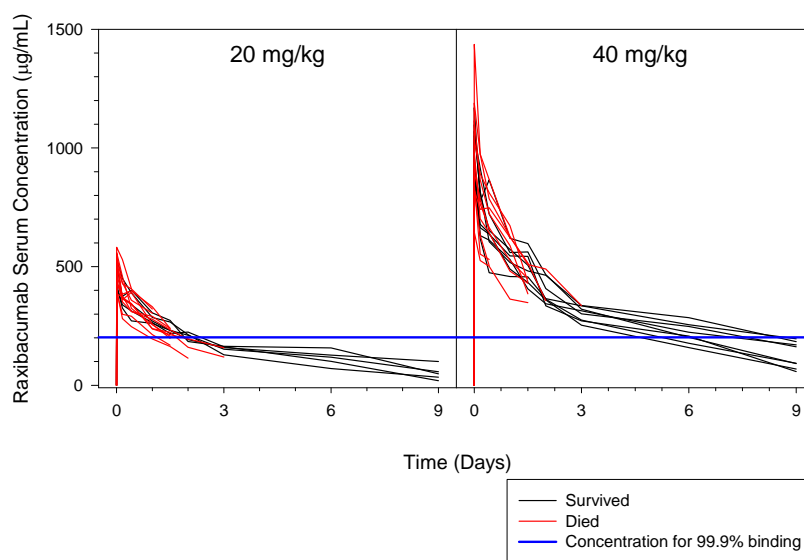
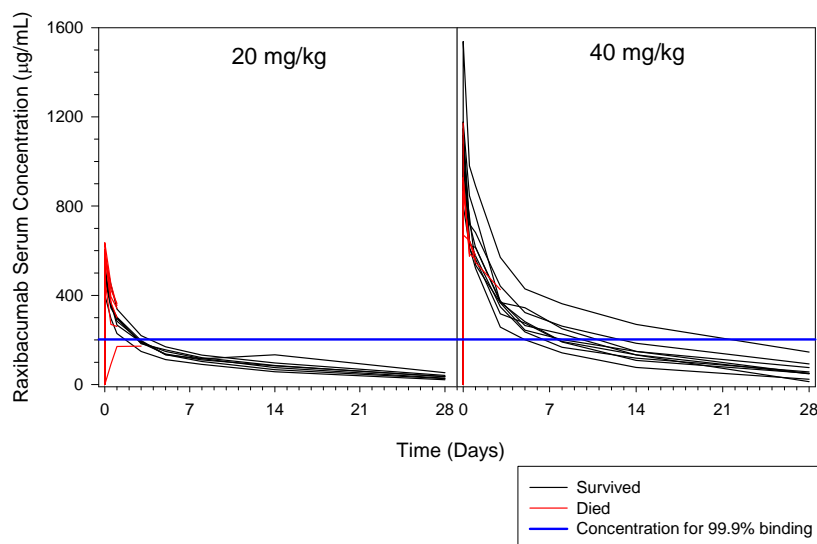


Figure 6.3-5: Spaghetti Plot of Serum Concentration-Time Profiles for Raxibacumab Following Single Intravenous Administration of 20 mg/kg and 40 mg/kg Raxibacumab in Spore-Challenged Monkeys (FDA analysis)



6.4 Bioanalytical Assessment

During the previous review cycle for raxibacumab, upon inspection the FDA identified deficiencies in the assays used to measure raxibacumab and ciprofloxacin plasma concentrations, which in turn caused reliability of the resulting pharmacokinetics to be in question. The complete response letter issued during the first review cycle requested that the applicant revise the analytical procedures to address the deficiencies, re-analyze the pharmacokinetic samples obtained from the human studies, and re-calculate pharmacokinetic parameters from these re-analyses. The FDA and the applicant agreed on “equivalence” criteria to compare the results obtained with the original assays with the results obtained with the modified assays for the purposes of supporting data integrity.

The applicant complied with the requests in the complete response letter, and upon review of the resubmitted data, FDA agrees that the pharmacokinetic data obtained by both the original and modified bioanalytical methods should be considered reliable. Therefore, the pharmacokinetic data for both raxibacumab and ciprofloxacin obtained by the original bioanalytical methods will be used for purposes of review conclusions and labeling.

6.5 Pediatric Dosing Recommendations

Based on rabbit and monkey PK/PD and efficacy studies and human PK and safety studies, the applicant concluded that the 40 mg/kg raxibacumab IV dose can provide adults with the desired level of protection against lethality. The PK and safety profile of 40 mg/kg IV raxibacumab in healthy adult humans was characterized in 4 clinical studies as previously described. The objective of the analysis described below was to derive pediatric doses for raxibacumab to match the adult PK (AUC and C_{max}) given 40 mg/kg of raxibacumab.

Methods

Pharmacokinetic data for this analysis were collected from 322 healthy adults in a total of 3 clinical studies (one of the four previously mentioned studies was conducted with a previous version of the drug product and was therefore not included in this analysis). NONMEM version VI was used for pharmacokinetic modeling and simulation. Splus version 6.2 and EXCEL were used for data formatting,

plotting and simulation. In order to derive pediatric data, clearance and body weight relationship from other monoclonal antibodies (e.g., canakinumab²¹, infliximab²², pertuzumab²³, trastuzumab²³) was investigated. It was consistently observed that clearance of monoclonal antibodies is strongly related to body weight.

Simulations were performed for a population with body weight range from 5-100 kg at dose 40 mg/kg. The predicted AUC and Cmax were compared with those observed in healthy adults with body weight > 45 kg. The 90% prediction interval of AUC and Cmax for adults was comparable with those seen in adults at 40 mg/kg. However, 40 mg/kg dose does not provide similar exposure for patients with body weights less than 45 kg. To match the exposure level in pediatric population to that in adults, several dose regimens for pediatric population with lighter body weight were simulated, as shown in Table 6.5-1. In the base case scenario, the IV dose is 40 mg/kg for all subjects in the simulations. In scenario I and II, the dose was adjusted for subjects with different body weights.

Table 6.5-1: Dosing Scenarios Simulated (FDA analysis)

Scenarios	Dosing Regimen	
Base case	40 mg/kg for all subjects	
Scenario I	WT (Kg)	Dose (mg/kg)
	≤5	100
	>5 to ≤ 10	80
	>10 to ≤ 15	70
	>15 to ≤ 35	60
	>35 to ≤ 50	50
Scenario II	WT (Kg)	Dose (mg/kg)
	≤ 15	80
	>15 to ≤ 50	60
	> 50	40

Results

The pediatric dosing regimen proposed by FDA was accepted by the applicant (see Table 6.5-2 below). As shown in Figure 6.5-4 and

²¹ http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/125319s000_ClinPharmR.pdf

²² Baldassano et al., “Infliximab Therapy in the Treatment of Pediatric Crohn’s Disease” *The American Journal of Gastroenterology* (2003) **98**, 833–838, <http://www.nature.com/ajg/journal/v98/n4/full/ajg2003198a.html>

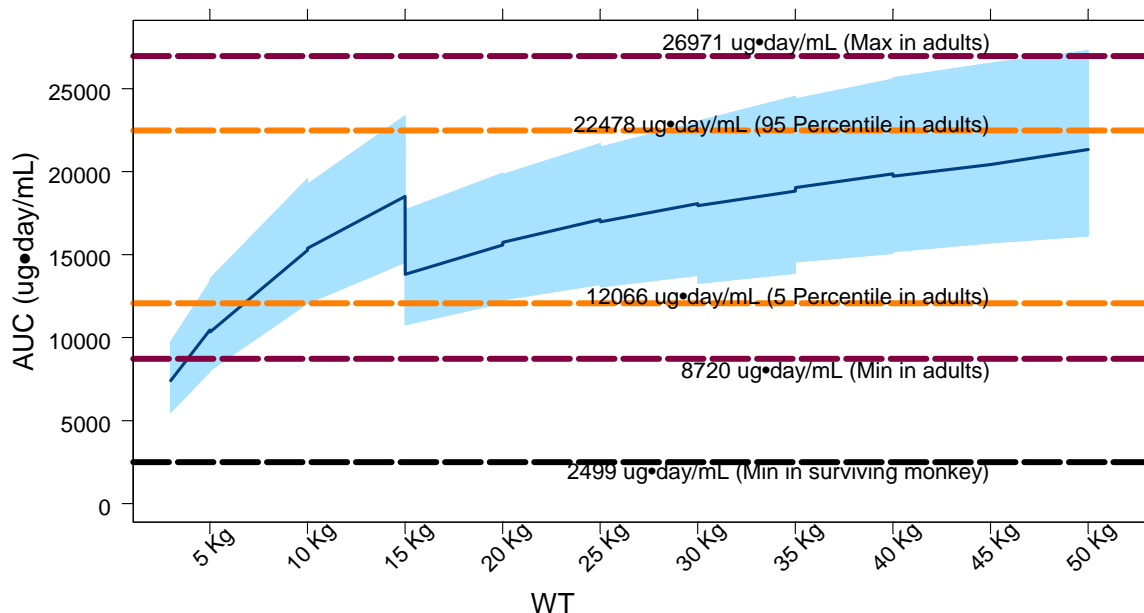
²³ Deng et al, Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: What have we learned?” *mAbs*, (2011) **3**:1,61-66

Figure 6.5-5, the proposed dosing recommendations reasonably match AUC and C_{\max} in healthy adults after 40 mg/kg dose. The proposed dosing is also simple to implement in emergency settings.

Table 6.5-2: Raxibacumab dosing recommendations for pediatric patients based on body weight (WT) (FDA analysis)

WT (Kg)	IV Dose (mg/kg)
≤15	80
>15 to ≤50	60
>50	40

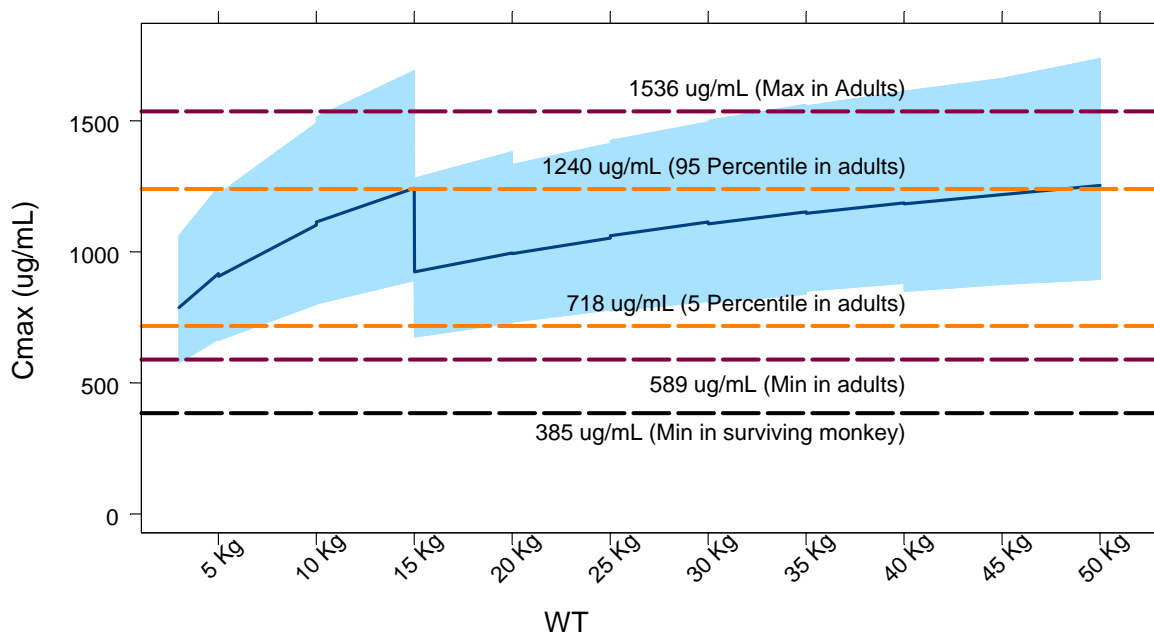
Figure 6.5-4: Predicted raxibacumab exposures (AUC) at the proposed dosing recommendations in Table 6.5-2: The solid blue line and shaded area represent median and predicted 5th, and 95th percentile of raxibacumab AUC in the pediatric population. The dash lines represent the range (minimum and maximum), 5th and 95th percentile AUC for adults (predicted from posthoc estimate by sponsor) at 40 mg/kg dose^a and minimum observed AUC in surviving monkeys^b at 20 mg/kg (FDA analysis)



a. From Summary Table 9, HGS1021-POP01.PK in Clinical Pharmacokinetics Report

b. From Appendix 37, HGS1021-POP01.PK in Clinical Pharmacokinetics Report

Figure 6.5-5: Predicted raxibacumab C_{max} at the proposed dosing recommendations in Table 6.5-2: The solid blue line and shaded area represent predicted 5th, median and 95th percentile of raxibacumab C_{max} in pediatric population. The dash lines represent the range (min and max), 5th and 95th percentile C_{max} for adults (predicted from posthoc estimate by sponsor) at 40 mg/kg dose^a and min observed C_{max} in surviving monkey^b (FDA analysis)



- a. From Summary Table 9, HGS1021-POP01.PK in Clinical Pharmacokinetics Report
- b. From Appendix 37, HGS1021-POP01.PK in Clinical Pharmacokinetics Report

7 Safety

7.1 Nonclinical Pharmacology/Toxicology Safety

7.1.1 Tissue Cross-Reactivity of Raxibacumab

Two tissue cross-reactivity studies with raxibacumab showed an absence of positive staining in a majority of donor tissues from human, cynomolgus monkey, and New Zealand White rabbit at two different antibody concentrations. In particular, no tissue cross-reactivity was observed in the brain (cerebrum and cerebellum) or spinal cord of any species examined. However, cross-reactive binding with PA mAb was evident in the thyroid in both studies, showing strong, punctate staining of cytoplasmic granules in follicular epithelium. In Study 1494-95 (2003), positive staining of thyroid tissue was noted to be greater in monkeys than humans, whereas in study no. 1M1634 (2008), equally strong antibody binding in both human and monkey donor thyroid tissue was detected with complete absence of tissue reactivity in the rabbit. Weak, inconsistent staining of skeletal muscle (both humans and monkeys), endometrium (humans only), and breast and prostate (monkeys only) tissues was also reported in the earlier study which was not observed in the study completed in 2008. The cause(s) for the difference in results between the two studies is unknown but may have resulted from the different manufacturing process used to generate each antibody.

(developmental process versus the to-be-marketed product). Alternatively, donor thyroid tissue from rabbit may contain specific or non-specific off-target binding sites different from monkeys and humans which prevented binding of human PA mAb in this species.

7.1.2 General Toxicity of Raxibacumab in Healthy Cynomolgus Monkeys

Healthy cynomolgus monkeys treated with raxibacumab (40 mg/kg) showed no significant evidence of toxicity in a 120-day repeat dose study (Study 6962-140). Raxibacumab was administered three times (once every 12 days) by either intravenous or subcutaneous injection (Phase 1) plus a single intramuscular injection (to both cohorts) on day 69 (Phase 2). All monkeys survived with no reported test article effects different from the control group.

No obvious sex-dependent differences in raxibacumab were observed. No immunogenic response above background levels was detected in healthy cynomolgus monkeys treated with raxibacumab in the 120-day study. Antibodies against the Fab and Fc portion of the PA mAb antibody in raxibacumab-treated animals failed to increase above control levels determined from normal sera from 50 untreated monkeys.

7.1.3 Reproductive Toxicology of Raxibacumab in Healthy New Zealand White Rabbits

Healthy New Zealand White rabbits treated with raxibacumab (40 or 120 mg/kg) showed no evidence of test-article related maternal or embryo-fetal developmental toxicity different from controls (Study 6962-173). Raxibacumab was administered on gestation day (GD) 7 and again on GD 14 by intravenous injection. Toxicokinetic analysis in raxibacumab-treated pregnant dams (n = 3 per dose) in the above mentioned study showed dose linearity across a 3-fold dose range. A trend towards a slightly lower serum concentration of raxibacumab in several animals with detectable levels of anti-PA mAb antibodies was detected at both doses. In addition, several animals negative for the anti-raxibacumab antibodies in the 120 mg/kg dose group also showed decreased serum raxibacumab levels, suggesting neutralization and/or assay interference due to serum raxibacumab concentrations in excess of 150 µg/mL. The raxibacumab concentration-time profiles in pregnant rabbits in this study were similar to those predicted from non-pregnant rabbits.

The overall incidence of anti-raxibacumab antibody response in pregnant NZW dams above background levels was very low (5/138). However, findings of anti-raxibacumab antibodies in an untreated control animal combined with high serum levels of raxibacumab > 150 µg/mL in the 120 mg/kg dose group may have reduced the sensitivity of the assay for the anti-PA mAb response. Of 11 samples from the 120 mg/kg group that exceeded the raxibacumab serum limit, seven were negative, four were potentially positive, and only one confirmed positive in the assay. Such potentially confounding results may preclude any findings and conclusions reported from this study.

7.2 Clinical Safety

A total of 326 healthy volunteers received the recommended dose of raxibacumab of 40 mg/kg manufactured by the M11 process (the to-be-marketed product) and were evaluated for safety of raxibacumab. Of these, 303 subjects received a single dose (Study HGS1021-C1064 and Study HGS1021-C1063), 23 subjects received a second dose two weeks after their first dose (Study HGS1021-1063), and 20 subjects from Study HGS1021-C1064 received a 2nd dose more than 4 months after their initial dose (Study HGS1021-C1069). In Study HGS-1021-C1064, 48 subjects who received raxibacumab also received ciprofloxacin as part of this drug interaction study.

Studies HGS1021-C1064, HGS1021-C1063 and HGS1021-C1069 are briefly summarized in the Table 7.2-1 below.

Table 7.2-1: Studies in Healthy Volunteers included in the Safety Analysis

Study ID	Design Control Type	Group: Entered/ Completed	Indication	Sex M/F; Median age (range); Race	Duration	Study and Control Drug, Route, Regimen, Dosing frequency
HGS-1021-C1063	P3, R, SB, PC, RD	Double dose raxibacumab:24/23 Double dose placebo: 8/5 Single dose raxibacumab: 216/206 Single dose placebo: 72/68	Safety and PK in healthy subjects with single and repeat injection	156M/164F; 39.8 (18-88); 259W, 30B, 24A, 13NL, 10MR, 5NH	Single-dose (day 0) Double-dose (days 0 and 14) with 56 day follow-up	Raxibacumab, IV Placebo, IV; Single dose (day 0), double dose (days 0 and 14)
HGS-1021-C1069	P2/3, SG, OL, RD	All 20 subjects treated with a single 40 mg/kg IV raxibacumab dose at least 4 months after an initial 40 mg/kg. Single dose administered IV during study HGS-1021-C1064	Safety and PK in healthy subjects with repeat injection	12M/8F; 38.5 (22-61); 13W, 7B, 4HL	Single-dose with 56 day follow-up	Raxibacumab, IV; single dose
HGS-1021-C1064	P2/3, R, OL, single-dose	PO cipro, IV raxi : 32/26 IV raxi: 28/22 IV cipro, IV raxi :28/22	Safety and PK in healthy subjects with concomitant use with ciprofloxacin	43M/45F; 34.4 (18-61); 53W, 31B, 3NL, 1BNH, 21HL	7 days with 56 day follow-up	Raxibacumab IV; Ciprofloxacin PO; Ciprofloxacin IV Raxibacumab IV single dose; Cipro PO Q12h, 15 doses, Cipro IV 2 doses 12h apart

A, Asian; AI, American India; B, Black; BNH, Black and Native Hawaiian; HL Hispanic or Latino; MR, Multiracial;

NH Native Hawaiian; NL Not listed; W, White

CPL, Cipro, Ciprofloxacin

OL, Open label; P1, Phase 1; P2/3, Phase 2/3; P3, Phase 3

P, Placebo; PC, Placebo-controlled; R, Randomized; RD Repeat dose; SB, Single blind; SG, Single Group; Tx, Number of subjects treated

Modified from original BLA Integrated Summary of Safety Table 1-1 pp. 18-19

Demographics for all 326 subjects are shown below in Table 7.2-2. Most subjects were white, within a median age of 38 years (range 18 to 88 years) with a similar number of males and females enrolled.

Table 7.2-2: Demographics of Subjects Enrolled Across Studies

	Single-Dose (HGS1021-C1063 + HGS1021-C1064) ¹ N = 283	Double-Dose ≥ 4 Months Apart (HGS1021-C1069) N = 20	Double-Dose 14 Days Apart (HGS1021- C1063) N = 23	All Raxibacumab Treated (HGS1021-C1064/ HGS1021-C1069 ² +HGS1021-C1063) N = 326
Sex				
Male	130 (45.9 %)	12 (60.0 %)	10 (43.5 %)	152 (46.6 %)
Female	153 (54.1 %)	8 (40.0 %)	13 (56.5 %)	174 (53.4 %)
Race³				
White	207 (73.1 %)	14 (70.0 %)	21 (91.3 %)	242 (74.2 %)
Asian	18 (6.4 %)	-	-	18 (5.5 %)
Black or African American	48 (17.0 %)	6 (30.0 %)	1 (4.3 %)	55 (16.9 %)
American Indian or Alaska Native	1 (0.4 %)	-	-	1 (0.3 %)
Native Hawaiian or Other Pacific Islander	5 (1.8 %)	-	-	5 (1.5 %)
Not Listed	14 (4.9 %)	-	1 (4.3 %)	15 (4.6 %)
Multiracial	9 (3.2 %)	-	-	9 (2.8 %)
Hispanic or Latino origin	46 (16.3 %)	3 (15.0 %)	1 (4.3 %)	50 (15.3 %)
Age (years)⁴				
n	283	20	23	326
Mean ± SD	39.0 ± 15.4	40.5 ± 13.3	48.5 ± 14.6	39.8 ± 15.3
Median	36.5	38.5	50.5	37.4
Range	(18.1, 87.9)	(22.4, 60.6)	(22.1, 76.2)	(18.1, 87.9)
Age group				
< 65 years	264 (93.3 %)	20 (100.0 %)	21 (91.3 %)	305 (93.6 %)
≥ 65 years	19 (6.7 %)	-	2 (8.7 %)	21 (6.4 %)
Weight (kg)				
n	283	20	23	326
Mean ± SD	76.3 ± 17.3	78.4 ± 13.9	82.6 ± 19.2	76.9 ± 17.3
Median	74.7	77.2	79.9	75.6
Range	(44.6, 155.9)	(56.5, 98.6)	(54.8, 121.6)	(44.6, 155.9)

¹ Excluded the 20 subjects who received a 2nd Raxibacumab dose in HGS1021-C1069.

² Subjects participating in both HGS1021-C1064 and HGS1021-C1069 were counted once only with race, age, and weight based on HGS1021-C1064.

³ Subjects who checked more than one race category were counted under individual race categories as well as the multiracial category.

⁴ Age was calculated from the date of birth to the date of randomization.

From original BLA Summary of Clinical Safety Table 2.7.4-3 p.24

Findings

Deaths

There was one death reported among the 326 subjects. Subject US001-002 was enrolled in study HGS1021-C1063 and randomized to the placebo double-dose group. This subject died from injuries sustained in a motor vehicle accident.

Serious Adverse Reactions (SAR)²⁴

Three subjects (0.9%) experienced a serious adverse reaction, which are shown in Table 7.2-3.

²⁴ Serious adverse reactions are defined as events that are life-threatening, resulted in hospitalization, death, permanent disability; a congenital malformation.

Table 7.2-3: SAR reported across all studies

Study	Treatment received	Subject ID	A/S /R	MedDRA system organ class	Preferred Term	AE Start Day/ Duration (days)	Outcome	Relationship to study drug	Action taken with study drug
HGS1021-C1063	Placebo double dose	US001-002	45/ F/W	Injury, poisoning and procedural	Injury	39/1	Fatal	Not related	None
HGS1021-C1063	Raxibacumab double dose	US002-017	52/ F/W	Hepatobiliary disorders	Cholecystitis	24/3	Recovered/ resolved	Possibly related	None
HGS1021-C1064	Raxibacumab single dose	US001-005	48/ B/ M	Psychiatric disorders	Schizophrenia	5/ ongoing	Not recovered/ not resolved	Probably not related	None

A/S/R: age/sex/race
FDA table

Both cases of cholecystitis and schizophrenia were deemed unlikely to be related to raxibacumab based on temporal presentation (long duration between raxibacumab dosing and onset of the AE) and the presence of pre-existing conditions which could account for the SAE.

Dropouts and Discontinuations

There were no subject withdrawals due to adverse reactions (AR). However, there were four subjects (1.2%) who experienced AR and discontinued treatment, as shown in Table 7.2-4.

Table 7.2-4: Subjects with AR Resulting in Discontinuation of Treatment

Study	Treatment received	Subject ID	A/S /R	Preferred Term	Serious/ Severity	Study Day	Relationship to study drug	Notes
HGS1021-C1064	Cipro/raxibacumab	US003-006	36/ F/W	Urticaria	No/mild	5	Probably related	No premedication with diphenhydramine
HGS1021-C1064	Cipro/raxibacumab	US003-028	56/ F/W	Clonus	No/mild	5	Probably related	“Left arm and leg clonic muscular contractions” and “facial flushing”, both mild and resolved in 1 day; no change in vital signs
HGS1021-C1064	Raxibacumab single dose	US003-002	33/ F/W	Urticaria	No/mild	1	Probably related	No premedication with diphenhydramine
HGS1021-C1063	Raxibacumab single dose	US002-011	26/ M/ B	Dyspnea	No/mode rate	0	Probably not related	“Difficulty breathing” of moderate severity during infusion; no sx anaphylaxis or rash, no changes in vital signs; investigator attributed the subject’s symptoms as most likely due to esophageal spasm caused by rapid eating prior to the infusion and/or anxiousness
HGS1021-C1063	Placebo double dose	US001-022	59/ M/ W	Skin infection	No/mode rate	12	-	

A/S/R: age/sex/race
FDA table

Adverse Reactions of Severe Intensity

AR of severe intensity were reported in 1.9% of raxibacumab-treated subjects and are shown in Table 7.2-5.

Table 7.2-5: Severe AR across Studies (FDA analysis)

MedDRA system organ class	MedDRA Preferred Term	Single Dose - C1063+C1064 N=283 (%)	Double Dose - C1063 N=23 (%)	All raxibacumab subjects* N=326 (%)
Hepatobiliary disorders	Cholecystitis	0	1 (4.4)	1 (0.2)
Infections and infestations	Influenza	1 (0.4)	0	1 (0.2)
Investigations	Blood amylase increased	1 (0.4)	0	1 (0.2)
	Blood creatine phosphokinase increased	1 (0.4)	0	1 (0.2)
	Prothrombin time prolonged	1 (0.4)	0	1 (0.2)
Psychiatric disorders	Schizophrenia	1(0.4)	0	1 (0.2)

*There were no AR of severe intensity reported in the double dose separated by ≥ 4 months group (Study HGS1021-1069) although they are included in the Total Subject count
FDA table

There were 2 severe and serious AR in raxibacumab-treated subjects (cholecystitis, schizophrenia) which were described previously. In placebo-treated subjects, there was a single event of severe leukocytosis reported. All other severe AR were unlikely to be related to raxibacumab.

Common Adverse Reactions

The most frequently reported system organ classes (SOC) for AR in the raxibacumab-treated subjects were nervous system disorders (13.2%), infections and infestations (11.3%), and gastrointestinal disorders and skin and subcutaneous tissue disorders (both 9.2%). These were all higher in the raxibacumab-treated subjects compared to placebo.

CNS Adverse Reactions

Based on the increased CNS findings in animals treated with raxibacumab that died of anthrax in the pivotal efficacy studies, CNS AR were examined more closely in the human safety database.

In the nervous system disorders SOC, headache was by far the most frequently reported AR. It was reported in 8.8% of single dose subjects, 14% of double dose subjects, and 10% of placebo subjects. The following nervous system disorders AR were reported (Table 7.2-6).

Table 7.2-6: Nervous System Disorders across all Studies (FDA analysis)

MedDRA system organ class	MedDRA Preferred Term	Placebo* – C1063 N=80 (%)	Single Dose - C1063+C1064 N=283 (%)	Double Dose - C1063 N=23 (%)	Double Dose - C1069 N=20 (%)	All raxibacumab subjects N=326 (%)
Nervous system disorders	Clonus	0	1 (0.4)	0	0	1 (0.2)
	Dizziness	1 (1.3)	3 (1.1)	0	0	3 (0.6)
	Headache	8 (10)	25 (8.8)	1 (4.3)	5 (25)	31 (9.5)
	Lethargy	1 (1.3)	0	0	1 (5)	1 (0.2)
	Paresthesia	0	1 (0.4)	0	0	1 (0.2)
	Somnolence	0	4 (1.4)	1 (4.3)	0	5 (1.5)
	Syncope vasovagal	0	2 (0.7)	0	0	2 (0.4)
	Tension headache	2 (2.5)	0	0	0	0

* includes both single (n=74) and double (n=6) dose placebo subjects

Headache was reported in similar percentages of placebo and raxibacumab treated subjects (10% and 9.5%, respectively). There were an additional two reports of tension headache in the placebo group.

There were no reports of meningismus across human safety studies. A single raxibacumab treated subject (US004-000030) enrolled in study HGS1021-C1063 reported fever (mild) on day 41 in conjunction with vomiting (mild), both of one day duration with no action taken. Similarly, there was one placebo subject US005-000046 enrolled in Study HGS1021-C1063 with pyrexia and headache reported on Study day 36, again of one day's duration with no intervention. It is unlikely that either of these represented cases of meningitis.

Infections/Infestations

In contrast, within the infections and infestations SOC, upper respiratory tract infection occurred in 11.6% of double dose raxibacumab subjects, compared to 3.9% of single dose raxibacumab subjects and 5.0% of placebo subjects. However, other infections were not reported more frequently in the active treatment groups to suggest a uniform immunomodulatory/suppressive effect of raxibacumab. All AEs in the raxibacumab treated subjects ranged in frequency from 0.2% to 4.9%.

Gastrointestinal SOC

In the gastrointestinal disorders SOC, rates of abdominal pain, diarrhea, nausea and vomiting were all higher in the placebo group compared to all raxibacumab subjects.

Dermatologic (Skin and subcutaneous tissue) SOC

Lastly, in the skin and subcutaneous tissue disorders SOC, AR reported by the raxibacumab-treated subjects ranged from 0.2% to 1.8% in frequency. Pruritis and rash were reported in all raxibacumab subjects at 2.1% and 1.8% respectively, and were comparable to placebo rates (pruritis 0%, rash 1.3%). In Study HGS1021-C1064 evaluating raxibacumab PK in combination with ciprofloxacin, five of the first 25 subjects dosed with raxibacumab developed dermatologic events following administration. Three subjects responded to oral diphenhydramine, and two discontinued the infusion with resolution (one with diphenhydramine and one spontaneous). As a result of these findings, the protocol was amended so that all subsequent subjects received diphenhydramine before raxibacumab infusion. At the completion of HGS1021-C1064 a total of 8/88 (9.1%) subjects reported rash. In comparison, rash occurred in 6/27 (22%) of a subset of subjects who did not receive diphenhydramine premedication. Furthermore, to closely match the anticipated use of raxibacumab in human inhalational anthrax, diphenhydramine pretreatment was also given to the monkeys participating in the inhalational anthrax efficacy studies.

Laboratory Findings

Laboratory tests with toxicity grade greater than or equal to 3 (severe) were infrequently reported. Overall, the incidence of laboratory abnormalities was comparable between raxibacumab-treated and placebo-treated subjects (only active treatment AR reported here). The incidence was not higher after a second raxibacumab administration than after a single administration. Most lab abnormalities were of low grade (Grade 2 or less), with grade 3 and 4 laboratory abnormalities infrequently reported, isolated and transient.

Special Populations

Adverse reactions were analyzed by sex, race and age, and no significant differences were found with these subgroup analyses.

7.3 Immunogenicity

Nonclinical:

No immunologic response above background levels was detected in healthy cynomolgus monkeys treated with raxibacumab in the 120-day study. Antibodies against the Fab and Fc portion of the PA mAb antibody in raxibacumab-treated animals failed to increase above control levels determined from normal sera from 50 untreated monkeys.

The overall incidence of anti-raxibacumab antibody response in pregnant NZW dams above background levels was very low (5/138).

Pivotal Animal Studies:

No immunogenicity assessments were performed in pivotal animal (both rabbits and NHP) efficacy studies.

Combination Animal Studies:

Anti-raxibacumab immunogenicity assessments were added to Study 781-G923701 (NZW rabbits) as a protocol amendment to allow for determination of the impact of any observed anti-raxibacumab immunogenicity on plasma raxibacumab levels and survival.

Fifteen of 18 rabbits (83%) in the levofloxacin/raxibacumab group tested positive for anti-raxibacumab antibodies on Day 28. In addition, 6/19 (31.6%) rabbits in the levofloxacin group also tested positive for anti-raxibacumab antibodies on Day 28. HGS accounted for this finding by noting that although the levofloxacin only treatment group did not receive raxibacumab, the levofloxacin-treated rabbits mounted an anti-PA response which may have included antibodies that cross-reacted in the anti-human anti-PA assay. Given the modest incidence of anti-raxibacumab immunogenicity at low titers compared with the raxibacumab-treated animals, this was felt to be a possible explanation. Furthermore, HGS pointed out that the emergence of potential anti-raxibacumab immunogenicity did not negatively impact survival.

Human

All four human safety studies looked for the development of an anti-raxibacumab antibody response, including PAM-NH-01 which used the developmental M10 formulation of the product. In Studies HGS1021-C1064, HGS1021-C1063 (second raxibacumab dose 2 weeks following first dose) and HGS1021-C1069 (second dose \geq 4 months following first dose, there were no subjects who had a positive anti-raxibacumab antibody response following single or repeat (every 14 days or following \geq 4 months) doses of raxibacumab.

8 Points for Discussion

Considering the information described for raxibacumab in the rabbit and monkey animal model studies and safety trials in healthy human volunteers:

1. Do the results from the therapeutic studies of raxibacumab with and without antimicrobials in two animal models of inhalational anthrax provide substantial evidence that raxibacumab 40 mg/kg IV single dose is reasonably likely to produce clinical benefit for the treatment of humans with inhalational anthrax?

If not, what additional studies are needed?

2. Do the results from raxibacumab safety trials in healthy volunteers and studies in animals support an acceptable risk benefit profile given the benefits of the therapy?

If not, what additional studies are needed?